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THE UNIVERSITY OF ALBERTA

A STUDY OF SECONDARY CLOSURE IN INFECTED WOUND HEALING

by

BRIAN WILLIAM JOHNSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL, 1983

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A STUDY OF SECONDARY CLOSURE IN INFECTED WOUND HEALING submitted by BRIAN JOHNSON in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

.....
Supervisor

.....

.....

.....

Dated May 27, 1983....

DEDICATION

To my wife, Brenda

A B S T R A C T

This research assessed the primary and secondary healing in infected wounds: an experimental study.

The beneficial effect of delayed closure of contaminated wounds is well established clinically; however, the morphology, breaking strengths and biochemical nature of these wounds have received little attention. 360 female Wistar rats (2-3 months old) were divided into groups for infection with E. coli or Staph. aureus or controls and into subgroups for primary or secondary closure. At 7, 14, 21, 42, 84, and 119 days, 10 animals from each subgroup were sacrificed and the wounds were subjected to breaking strength determination, hydroxyproline concentration analysis, histological examination including collagen and reticular fibre stains and scanning electron microscopy. In the control group the secondarily closed wounds were consistently stronger and on 4 of 6 testing dates these differences were statistically significant ($p < 0.05$). Infection with Staph. aureus was associated with stronger wounds than those of the primary control group independent of the closure method used. In the E. coli infected group, the primarily closed wounds were significantly weaker at 14 ($p < 0.01$) and 84 ($p < 0.001$) days than the secondary closure subgroup. Hydroxyproline concentration exhibited a rapid early rise, followed by a decrease and a plateau phase, and there were no apparent differences between groups or closure technique. Scanning electron microscopy proved to be unsuitable for demonstrating morphological differences between subgroups.

Histological examination showed wound immaturity, relative collagen disorientation and increased reticular fibre content in those wounds with evidence of ongoing inflammatory changes. A review of the animals' course showed a decreased incidence of inflammation, abscess formation, hernia and mortality in the secondarily closed infected subgroups. It is concluded that secondary closure has a promoting effect on wound healing in the presence or absence of infection and may negate detrimental effects of the latter.

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INTRODUCTION

A. General:

Because of the remarkable similarities between the processes of infection and wound healing, the study of the two occurring simultaneously is complex and has not been well defined. The orderly sequence of events in wound healing has been studied extensively in order to characterize and correct defects in repair and in the hope of finding means to accelerate the normal rate. The significant insult of bacterial contamination with alterations of the tissue environment can have multiple effects on wound healing.

The strength of a wound is perhaps the most apparent feature. The rate of repair as well as the continuing integrity of the wound are of paramount importance to the surgeon and his patient. It is of considerable interest that apparently divergent observations have been made on the effects of infection on wound healing.

B. Historical Review:

1. Clinical Wound Infections

Clinical studies have shown multiple deleterious effects of wound infections. Conolly et al [1] reported major complication in 10.5% of patients with moderately contaminated wounds as compared to a complication rate of 1-2% in patients with clean wounds. The most serious result of impaired healing is complete dehiscence of the

abdominal wound which often requires further anaesthesia and surgery. It has been estimated that probably 25% of abdominal dehiscences are infected or result from an infection in the wound[2]. The incidence of incisional hernias occurring after clean wounds is 2 to 5% but the incidence of hernia in infected wounds is about 25 to 40% [3,4].

Keill[5] reviewed 4,242 procedures and found 47 abdominal wound dehiscences; an incidence of 1.11%. In the group with dehiscences, 72.3% were found to have wound infections by clinical evidence and culture documentation. Anemia, wound contamination, age over 50 years, and vertical incisions were also found to be significant factors; whereas race, primary disease, type of closure, operator, re-operation, jaundice, use of steroids, obesity, increased intra-abdominal pressure and hypo-proteinemia could not be established as statistically significant factors in the cause of dehiscence in this study. Confirming the importance of wound infections, McVay commented on this study, stating "our worst hernias have developed in smoldering anaerobic wound infections beneath a neatly closed skin."

Schilling and Heimbach[6] noted that 9.9% of 1470 surgical wounds suffered some sort of complication which he defined as a "quantitative physiological variant". The most frequent wound complication in their series was infection which occurred in 40% of cases.

Renvall et al.[7] prospectively studied 696 operations. They found an overall infection rate of 9.8%. For clean wounds, the rate was 4.2%; for clean-contaminated cases - 9.1%; for contaminated cases -14.4%; and for dirty wounds -28.8%. Significantly, the rate of other

post-operative infections in patients without wound infections was 12.7% but in patients with wound infections the corresponding figure was 28%. The patients with infected wounds required an average 6.6 additional days of hospitalization and the mortality rose from 1.6% in clean wounds to 11.8% in patients with wound infections. This last figure must be interpreted with caution as cases which are more likely to become infected usually are associated with a higher mortality. It is also noted that wound infections per se were not a major cause of death but other types of septic complication as well as other complications such as dehiscence, resulting from the wound infection, were important determinants of mortality.

Haddad and Macon [8] retrospectively reviewed 18,180 abdominal procedures and found 70 cases of dehiscence and/or evisceration. They found a considerably lower mortality rate (5.5%) than generally reported and that the mortality rate in evisceration was considerably lower, being equal to that of dehiscence. The factor most frequently associated with wound dehiscence was exogenous obesity (40%). In decreasing frequency, they found prolonged ileus or bowel obstruction (35%), low albumin (33.3%) and wound infection (31.6%). However, they also included "dirty" procedures as being a predisposing factor which would increase to 45.6% the proportion of patients in which wound failure was associated with infection. The mortality in the wound infection group was negligible. This paper once again emphasizes the important relationship between wound infection and wound failure.

Alexander and Prudden[9] reviewed 200 wound disruptions which occurred at the Columbia Presbyterian Medical Center between 1945 and

1964. They concluded that wound disruptions occuring during the first three days postoperatively (11%) were due to technical error. They also noted that disruptions occurring beyond the eleventh postoperative day (6.5%) were asociated with high rates of complicating metabolic factors, such as infection (undefined), advanced age, and hypoalbuminemia. In this latter group, hypoalbuminemia, defined as less than 3.0 gm/100 ml, was seen in 71% and infection was observed in 31%.

In an eighteen month review of 2,253 consecutive general surgical cases at the Duke and Durham Veterans Administration Hospitals, Moylan and Kennedy [10] studied etiological factors in wound infections with particular attention to gown and drape barriers. Significant reductions in wound infection rate were noted with preoperative antibiotics (most commonly Keflin)† in clean-contaminated and contaminated cases but no benefit was apparent in clean surgical cases. Wound irrigation was found to reduce infection rates only in the contaminated group of cases. Longer operations were associated with a higher rate of infection. This could not be clearly ascribed to air-borne contamination since the influences of difficulty of operation, greater blood loss, and the continued effectiveness of the gown and drapery barriers are difficult to evaluate separately. The finding of pivotal interest in this study was that the use of the disposable spun-bonded olefin gown and drape system was associated with a lower rate of infections (2.27%) than the traditional cotton materials (6.41%).

†sodium cephalothin

2. Phases of Wound Healing

The processes of clean wound healing and their control have been reviewed by many investigators. Carpenter et al[11] outlined the normal uninterrupted course of wound healing in the four phases: hemostasis, acute inflammation, repair and consolidation. It was emphasized that the inflammatory process was a normal occurrence for wound debridement and that the inflammatory phase must begin to subside before repair can occur. The inhibitory effects on the wound healing process of infection and the acute inflammatory process were discussed. Resultant wound complications were noted to be related to wound infection.

Hunt and Van Winkle [12] discussed the progression of events in normal wound healing in terms of a module. The macrophage plays a central role by debriding the tissue, attracting other macrophages by chemotaxis, and apparently stimulating fibroblasts and promoting neovascularization. These cells secrete lactic acid which may stimulate collagen synthesis by fibroblasts [13]. The concept of these reparative processes being sequential and occurring asynchronously from the edge of normal tissue and gradually progressing into the wound defect as limited by vascularization and cellular debridement is well developed.

Schilling[14] noted that the traditional phases of wound healing (lag phase, logarithmic phase and waning phase) more appropriately apply to the sigmoid curve of tensile strength of wounds rather than the cellular events which are accordingly divided into wounding, inflammatory and remodelling phases. It is during the wounding phase

that bacterial contamination occurs and infection may be controlled by debridement, sterile technique, aspiration, irrigation, as well as the possible judicious use of peri-operative antibiotics. During the inflammatory phase the leukocyte infliltration is coincidental with bacterial replication. Organism type, virulence and concentration, together with host defence mechanisms are factors which combine to determine the course of development of a wound infection. During the proliferative phase, granulation tissue fills the defect of the wound. Granulation tissue is an admixture of varying proportions of capillaries, fibroblasts, transudate and inflammatory cells. It acts as a barrier to bacteria and provides a surface over which epithelial cells may advance. The remodelling phase is marked by a gain in tensile strength together with a decease of total collagen. This apparent discrepancy is explained by structural realignment of collagen fibres parallel to physiological tension lines with increasingly firm chemical cross-linking.

3. Wound Collagen Structure and Metabolism

In a review of wound healing research, Heughen and Hunt [15] noted the characteristics of collagen are largely dictated by its molecular structure and there is little species variation. Therefore, observations on laboratory animals can be extrapolated to man.

A recent review by Prockop[16,17] et al. discusses collagen classification and the stages in collagen synthesis. Collagens contain a triple helix composed of a characteristic arrangement of amino acids including 33% glycine, approximately 10% proline, 10%

hydroxproline and variable amounts of hydroxylysine.

At present, five isotypes of collagen molecules have been characterized. The amino acid sequence within the chains and the composition of chains within the triple-helix are distinguishing features. Type I collagen consists of two α 1 (I) chains and one α 2 (I) chain and is commonly found in skin, bone, tendon, and cornea. A type I trimer consisting of only α 1 (I) chains has been identified in certain culture cells and tumors. Type II collagen consists of three α 1 (II) chains and forms the bulk of cartilagenous collagen. Type III collagen is found mainly in fetal skin, blood vessels, the parenchyma of visceral organs, and to a lesser degree, adult skin. It is composed of three α 1 (III) chains and it is generally considered to be the reticulin described by histochemical techniques. Type (IV) collagen contains three α 1 (IV) chains or three α 2 (IV) chains which are notably larger than other collagen chains, and it is found in basement membranes. Type V collagen is present in small amounts in skin, smooth muscle, placenta and bone. It is composed of two α1 (V) chains and one α 2 (V) chain.

The steps in collagen biosynthesis can be summarized: translation of collagen mRNA, post-ribosomal intracellular modification (hydroxylation, glycosylation, and chain folding to form the triple helix), and extra-cellular events (procollagen processing, fibre assembly and cross-linking).

Disturbances of these orderly processes can markedly affect collagen. It follows that wound healing and the tensile strength of tissues may also be altered as both are a function of collagen

structure. It has been noted that patients with type IV Ehlers-Danlos syndrome [18] lack type III collagen and are found to have decreased tensile strength of skin, aorta, and intestinal tissues. Marked inhibition of prolyl hydroxylation prevents synthesis of a functional protein and inhibition of cross-linking has a detrimental effect on collagen tensile strength. Subtle alterations in the amount of hydroxylysine or glycosylated hydroxylysine may affect fibril formation. In wound healing prolyl hydroxylase activity is increased early and the collagen deposited resembles that of fetal tissues, in that the ratio of type III to I is high, the cross-links are immature and the hydroxylysine content of collagen is high.[18]

The results of Madden and Smith[19] support the concept of the "wound module". These investigators compared the rates of collagen synthesis in primary and dehisced, resutured wounds by measuring incorporation of radio-labelled proline into hydroxyproline. Collagen deposition in resutured wounds was detectable immediately in contrast to the situation in the primary wound where certain vascular and cellular conditions must first be established, giving rise to an apparent lag phase of about 48 hours.

Madden and Peacock [20] found the net rate of collagen deposition in rat cutaneous wounds to be maximal around the fourteenth day and that there was a correlation of new collagen deposition with gain in tensile strength through the first 10 weeks of healing. It is of interest that the specific activity of the radioactive hydroxyproline began to decline during the logarithmic phase of tensile strength gain. It must be assumed that the character or arrangement of the

newly deposited collagen or of the existing wound collagen must change to account for this progresive gain in tensile strength which plateaus at the thirteenth week. They acknowledge this discrepancy in a later paper [21] and note that the rate of new collagen deposition remains elevated above control levels during the time of gain of tensile strength.

Cohen et al.[22] reported an interesting finding in rat open skin wounds. In analysing punch biopsies and subsequent wound tissue they found significant incorporation of radioactive proline during the first three days, a period which has traditionally been thought to be lacking any collagen synthesis. By selective analysis, they found that this process occurred in the panniculus carnosus. It was postulated that this collagen synthesis is performed by pre-existing local fibroblasts in response to hypoxia or lactate. Significant fibroblast infiltration is not noted until day six in dermal wounds. The collagen type in this open wound model is not known. It is to be remembered that little tensile strength is gained during this phase, therefore it may be that the function of this collagen is one of scaffolding for the granulation tissue and subsequent wound collagen.

The role of the macrophage in the wound healing process was studied by Leibovich and Ross[23]. By causing macrophage depletion with an antimacrophage serum and inhibiting macrophage migration with hydrocortisone acetate, they found inhibition of wound debridement, delayed fibroblast appearance and reduced fibrosis. Another study [24] investigated the role of the macrophage in angiogenesis. Macrophages taken from a three week old wound were transplanted to a

two day old wound. Angiogenesis was found to occur four days earlier than in control animals which received no macrophages.

Myers and Rightor [25] were also able to accelerate wound healing, as measured by wound tensile strength, by injecting autogenous blood into the incision site 2 to 7 days prior to surgery. This process resulted in an inflammatory reaction with accompanying capillary and fibroblast proliferation. While fascial wounds showed nearly twice the tensile strength seen in control animals at four days, no differences were noted at ten days. The investigators concluded that this procedure induced a benign inflammation which seemed to augment the strength of the early wound.

The precise effect of oxygen in wound healing has not been well defined. Stephens and Hunt [26] found that varying the ambient oxygen tension directly affected the strength of wounds, but Kirk and Irvin[27] found that increasing the ambient oxygen tension had no beneficial effect on the abdominal closure, colon anastomosis or the ischemic colon anastomosis in rats. It was postulated that any benefit on the abdominal closure may have been negated by the additional trauma of the colon surgery.

Forrester et al. [28] performed a comparative study on tape-closed and sutured wounds in the same animal. They measured the biomechanical parameters of tensile strength and energy absorbance (elasticity) as well as measuring total hydroxyproline and performing light and scanning electron microscopy on the specimens. It was found that the tape-closed wounds had a greater tensile strength but that this apparently beneficial effect was accompanied by greater

"brittleness" which is likely to be deleterious. The amount of collagen on a microgram per gram wet weight basis was the same in both groups but it is noted that assaying dry tissue should give a more useful index of collagen content. The morphological studies showed a more organized fibre sturcture present in the tape-closed wounds and it was assumed that this feature was responsible for the differences in physical properties.

Shephard[29] compared the differences in tensile strengths between delayed primary closure and primary closure in rats. He found that a three to four day delay in closure resulted in a comparably strong dermal wound.

To study collagen types in the early wounds of children Gay et al.[30] implanted viscose cellulose sponges into the wounds at the end of surgery and harvested these 24 to [illegible] hours later. Specific immunofluorescence techniques were used to study the distribution of collagen types I and III. It was found that type III collagen appears early and its staining intensity increased up to 96 hours whereas type I collagen was not detected until 72 hours and increased gradually thereafter. It was postulated that type III collagen synthesis corresponds with the "lag" phase in "open" granulomas and skin wounds and that this type of collagen may not be responsible for any significant part of wound strength during the first postoperative days.

Clore et al.[31] analyzed the collagen types synthesized in early rat skin wounds by incubating biopsies in tissue culture medium with radioactive hydroxyproline, extracting the collagen and then

separating the collagen types. They found that type III collagen synthesis was significantly elevated at 10 hours but by 24 hours had returned to normal skin levels (20%).

4. Experimental Wound Infection

It has been documented in clinical series that wound infections appear to have acute and chronic deleterious effects on the patient. Assessment of alterations to wound healing caused by infection is therefore of considerable interest and practical difficulty. Since wound healing and infection both exhibit inflammatory changes, it is difficult to characterize the morphology of the wound when these events co-exist.

Hunt et al.[32] approached this problem by using a rabbit model with steel mesh cylinder implants into which bacteria were innoculated. They found that the bacterial count varied considerably within individual animals. The connective tissue inside the cylinder in the infected wounds was dense, friable, and edematous. The number of bacteria required to produce an infection was not constant but depended on several factors (tissue trauma, hematoma, host resistance, etc.) and no level of innoculation resulted in 100% infection.

Using the same model, the influence of distinct trauma in the form of severe muscle contusion was studied in conjunction with wound contamination[33]. The trauma caused increased blood viscosity and decreased concentration along with a doubling of wound infections. The addition of low molcular weight dextran infusion reversed the effects on viscosity and infection rate. It was proposed that the

wound susceptibility to infection was probably due to decreased nutritive blood flow and that maintainance of intravascular volume may reduce wound infections.

Niinikoski et al[34] studied the respiratory gas tensions and collagen in infected wounds. Viscose cellulose sponge implants were introduced into subcutaneous tissues through a dorsal midline incision in Sprague-Dawley rats. Five days after surgery the experimental group were infected with Proteus mirabilis. Aspiration sampling for respiratory gas tensions and collagen synthesis were then performed. The oxygen tensions gradually rose after the fifth day in the control group but the pO_2 in the infected wounds fell to zero mm Hg and remained at this level until the end of the experiment at 30 days. The pCO_2 levels in both groups were similar but the pH in the infected group rose, presumably due to the increased activity of the Proteus mirabilis with resultant ammonia production. Infection caused a decrease in the total amount of collagen but did not appear to affect the solubility of this protein. Possible explanations for the decreased oxygen tensions include consumption by the bacteria or inflammatory process or interference with local circulation. The unexpected finding of normal pCO_2 levels in the infected wounds suggested that local metabolism was not increased. The fall in the total weight of collagen produced was thought due to hypoxia.

A further paper[35] examined the effect of varying ambient oxygen concentrations on the development of wound infections. Rabbits with wire mesh cylinder implants were infected with Pseudomonas aeruginosa. At 11, 18, and 25 days, the hypoxic (10% oxygen) group

had significantly more infections than control animals, whereas, at 7, 11, 18, and 25 days, hyperoxic (40% O_2) animals had significantly fewer infections than controls. It was postulated that the changes in oxygen concentration probably influence host defence and suggested that moderate hypoxia increased the susceptibility to infection.

In a comprehensive study, Bierens de Haan et al.[36] investigated the role of infection and inflammation on wound healing. They induced distant aseptic muscular abscesses with turpentine in one group of rats, then 8 and 12 days later performed an operation consisting of gastric and abdominal wall incisions with a peritoneal defect. Other groups underwent the same operation but had pre-operative abscesses, intraoperative bacteremia (confirmed by blood culture), or local wound contamination with Pseudomonas aeruginosa. The group with the distant turpentine abscess showed significantly decreased bursting strengths of both the gastric and abdominal incisions. It is of interest that drainage of the sterile abscess at the time of operation prevented the occurrence of weaker wounds. The groups with distant Ps. aeruginosa abscesses and transient bacteremia showed decreased bursting strength but only when a high dosage of bacteria was used. When low conentrations were used, an increased bursting strength was noted. The authors were surprised to find no alteration of the bursting strength of the locally contaminated wounds at five days and it was noted that these wounds were grossly purulent. Hydroxproline content of a 1 cm square of tissue with the central wound was determined. The incorporation of a large amount of surrounding normal tissue may have influenced these results. They found decreased hydroxyproline

concentrations only in highly infected animals and in a protein starved subgroup. When a correlation between bursting strength and hydroxyproline content could not be made, they postulated that hydroxproline might be measured from "poor quality" collagen or lysed collagen molecules.

Smith and Enquist[37] performed a study on the effect of a standardized non-fatal staphylococcal wound infection in previously closed musculofascial incisions in Wistar rats. The wounds were studed at fourteen intervals between zero and fifty-six days for wound breaking strength, hydroxproline content, hexosamine content and histological appearance. It was found that the breaking strengths in the infected group were significantly decreased after the fourth day and it was thought that maximal strength was still not reached by day 56. It was of interest to note that the infected wounds underwent a gain in strength after day 35 which paralleled the gain in control animals from the sixth to fourteenth day. In the control animals the hydroxyproline content rose rapidly until day 14, after which it was remained relatively constant (while the breaking strength increased dramatically) and then gradually diminished. In the infected animals there was significantly more hydroxproline after the seventeenth day which persisted throughout the remainder of the testing period. The hexosamine content of the infected wounds remained significantly above the control levels on every test day. They concluded that there may be local inhibitory factors which decrease as the infection subsides and there may be a systemic effect by which collagen synthesis is

increased. They concluded that collagen quantity did not correlate well with wound strength.

In a series of four articles from the Methodist Hospital and State University of New York the effect of gram negative infections was studied. In the first paper[38] the effects of Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa and Streptoccocus fecalis, both individually and in various combinations, were studied in primarily closed musculofascial laparotomy incisions in the Wistar rat. Animals were sacrificed on the fourteenth day. In all but one of the fifteen combinations of gram-negative organisms, the wounds showed statistically significantly increased tensile strengths in comparison to a control group. Their technique of wound tissue harvest for hydroxproline included one cm square of tissue which, as previously noted, incorporates considerable normal tissue in the sample. Their hydroxproline measurements showed no positive correlation to breaking strength nor was there any significant difference compared to the control group. Histological assessment of the tissues showed considerably normal scar tissue together with purulent material in the experimental animals. The authors concluded that the marked inflammatory reaction was responsible for the increased strength of the wounds and that this reaction perhaps results in a locally hypermetabolic environment with increased collagen production.

A similar model[39] was modified with a subcutaneous wire mesh cylinder to allow for aspiration of wound fluids for measurement of pH, pO_2 and pCO_2. The animals were contaminated with sterile broth,

E. coli and heat-killed E. coli. At fourteen days the E. coli contiminated group showed statistically significantly increased breaking strength compared with the other two groups. The pH and pO_2 of the wound fluids was also significantly lower and the pCO_2 significantly higher in this same group. They concluded that infected wounds had increased vascularity with more oxygen release because of the acidosis but increased oxygen utilization. Because of the increased metabolism, more carbon dioxide would be subsequently released.

The same model was used to study the effect of varying the amount of the E. coli innoculum[40]. At fourteen days, wounds innoculated with 10^7 to 10^9 organisms were significantly stronger whereas those innoculated with 10^3 to 10^6 were stronger but not consistently so.

The final paper [40] in this series examined the timing of increased wound strength in the same model using E. coli and Ps. aeruginosa with sacrifice at 3, 7, 10, 14, and 21 days. Histologically the three day old infected wounds showed a more marked polymorphonuclear leukocyte infiltration into surrounding muscle, more congested capillaries and a wider zone of pus and granulation tissue. At seven days, the edema and inflammatory infiltration persisted and more fibroblasts and congested capillaries appeared. At the later dates, the infected wounds showed a peristence of increased fibroblasts and more dense, newly formed, broad collagen fibres which were not seen in the controls. The breaking strengths of the infected wounds were similar to the control group until day 14 at which there was a significant increase which persisted at day twenty-one. It

should be noted that quantitation of fibroblasts is prone to error because of identification problems with two-dimensional slides.

A preliminary report by Gruber et al.[42] showed a wound-healing accelerating effect of Staphyloccus aureus which appeared greatest during the first week and remained significantly increased at 2 1/2 weeks.

Bucknall[43] used a similar model to study bursting strength, hydroxyproline content, histology, fibroblast proliferation, and vascular changes. Animals were contaminated with Staph. aureus, Pseudomonas aeruginosa and a combination of E. coli and Proteus mirabilis and sacrificed at days five and eleven. Contrary to the previous reports the control group were significantly stronger ($p<0.001$) than any of the infected groups. On day seven, the hydroxyproline levels in all infected groups were found to be slightly above the controls. On histological examination, the fibroblasts were reported to be relatively few and the collagen to be organized in a "whorled" rather than linear pattern as seen in the controls. The number of labelled fibroblasts, endothelial cells and monocytes were decreased in the infected groups. Angiography showed marked distortion of vessels and thrombus formation with non-filling in the infected group as opposed to wound bridging by capillaries in the control animals.

As discussed earlier, the management of contaminated surgical wounds remains a problem with a significant morbidity and mortality. The pathogenesis of wound infection remains a balance between the contaminating organism, the host, and local wound defenses. It is

apparent that the number and virulence of bacteria are important variables in wound infection. Acute alterations of significant injury such as hypoperfusion, anoxia and hyperviscosity, all predispose to infection. Wound healing is impaired in the presence of severe protein deficiencies and malnutrition, vitamin C deficiencies, extremes of age, corticosteroids, cytotoxic agents, anticoagulants, diabetes, and irradiation[44]. In these instances wound infections are more likely to occur. Local factors which may predispose to infection are: traumatic surgical technique, lack of hemostasis, dead spaces and improper suture choice. Two review articles[1,11] emphasize the importance and efficacy of delayed primary closure in the wound which is prone to infection. It is common for wound infections to develop in the fatty subcutaneous tissues and a delay, which allows for granulation tissue to infiltrate, appears to discourage wound infections. This protective effect of granulation tissue was shown by Duproctier[45] in an elaborate study in 1933. Guinea-pig incisions were contaminated with a standardized broth of Staph. aureus at the time of operation and then 2, 6, 12, 24, 48, 72, 96, 120, and 144 hours after operation. The percentage of wound infections fell dramatically with the delay of innoculation.

An interesting but somewhat contradictory study in contaminated wound management was performed by Edlich et al.[46]. In the first part of their study they made paired dorsal skin incisions in guinea pigs and contaminated one of the wounds with Staph. aureus. Delayed primary closure of both wounds was done at one to seven days. The

infection rate was found to be 73% after a one day delay and this decline to 0% after a four day delay.

The second and seemingly conflicting study involved paired incisions with one wound primarily closed with tapes. Both the open and closed wounds were innoculated with the same organisms 24 to 124 hours post-operatively. Surprisingly, a greater proportion of the open wounds showed gross evidence of purulent infection and greater adjacent skin erythema. The investigation inferred that fluid accumulating within a closed wound may have bactericidal properties. It should be noted that neither bacteriological nor histological examinations of the compared wounds were made and the only criteria of infection which were applied were related to gross surface appearance.

The technique of delayed primary closure began to gain clinical popularity with the advent of Steri-Strip^{R} (3M Company) tapes. In a restrospective study of one surgeon's practice, Verrier et al.[47] found reductions of infection rates from 4.8% to 1.1% in clean-contaminated cases; 11.1% to 4.8% in contaminated cases; and 33% to 6.6% in infected cases. In a review of soft-tissue injuries in the Vietnam war, Heaton et al[48] found a 2.58% infection rate in 349 delayed closures.

Many[49,50,51,52] other papers confirm favourable effect of delayed primary closure on infection-prone wounds in clinical practice.

C. The Present Study:

In summary, the previous literature documents the deleterious

effects of wound infection on the patient. These effects are noted to be both local (wound dehiscence, herniation) and systemic (associated distant infections, increased morbidity, and increased mortality). Although not uniform, experimental studies report contradictory findings in infected wound healing. Wounds have been reported to have accelerated and greater tensile strengths when infected. It is relevant to note that these experimental studies were all relatively short-term with no consideration of the chronic fate of the wound.

Delayed closure has been proven as a useful adjuvant in the management of infected wound healing. Clinical acceptance, however, has not been universal. Objections to delayed closure may be centered on aesthetic considerations. Some surgeons are reluctant to leave the skin edges unopposed at the completion of operative procedures. If uninformed, patients may be surprised and upset at the sight of a gaping surgical wound. Interestingly, patient objection or the potential of the same may play a considerable role in the surgeon's choice of closure. In the Unites States, which has major medico-legal problems and a large proportion of private patients, it has been estimated that only 10% of surgeons practice delayed closure. This is in contrast to the United Kingdom where medico-legal issues and private patients are considerably less prevalent and delayed closure is more often performed.

Delayed closure is technically simple but poorly understood physiologically. It is known that delayed closure may decrease the infection rate in contaminated wounds and minimally alters wound

tensile strength in clean wounds, however, other parameters have not been studied.

A general clinical impression, which is supported by many experimental studies, is that healing of infected wounds is impaired. Clinical wounds that are known to be contaminated have a decreased incidence of the manifestations of infection if allowed to heal by delayed primary or secondary closure.

As discussed, the physiology of secondary closure is poorly understood and has not been investigated in infected wounds. Whereas, secondary closure has a relatively predictable effect on the infection rate of wounds, an extensive review of the literature failed to reveal any studies of the strength of secondarily closed fascial wounds.

This study is designed to compare breaking strengths of fascial wounds in a variety of situations. First, the strength of primary and secondary closed clean wounds are examined acutely and chronically. Second, the same investigation is performed in wounds contaminated with a common Gram-negative (E. coli) or Gram-positive (Staph. aureus) organism. Further study of the wound tissue includes histology (with specific examination of inflammatory changes, collagen and reticular tissue staining, and collagen alignment and thickness on scanning electron microscopy) and collagen concentration.

Assessment of secondary closure in this manner should advance the understanding of wound healing in an infected environment.

MATERIALS AND METHODS

A. The Experimental Animal:

The Wistar rat (supplier: Woodbyn Laboratories, Guelph, Ontario) was chosen as the animal model. As noted previously [15] collagen synthesis in the animal model can reliably be correlated with that in the human. The Wistar breed was selected because of its hardy nature which was considered to be advantageous in a study employing deliberate infection[27,37-41]. Many of the previous studies[19,22,25,28,29,31,34,36,42-43] used the Sprague-Dawley rats. The local experience shows that this animal is more prone to respiratory infections and that clean laparotomy incisions are somewhat more frequently infected. Because these phenomena might have introduced significant variables into an infected wound healing experiment, it was decided to use the Wistar rat.

Once the animals were received, they were caged individually and observed for a minimum period of two days. This was done to determine if there was any underlying illness that became apparent following transport or if the animals had acquired any illnesses during transport.

Prior to and following surgery, the animals were maintained in individual cages with separate food and water sources. The rats received a diet of Wayne Lab-Blox pellets (Allied Mills, Chicago, Illinois) and water ad libitum. Environmentally, the animals had 100% room air and 100% ventilation. The ventilation allowed for 15 air changes per hour which is considered a requirement of metabolically

active animals, epecially with the additional stress of surgery. The temperature was maintained at 20±1°C. The vivarium had no external windows and timed fluorescent lighting was provided for 12 hours daily. Humidity was maintained at 40-60%.

All animals weighed between 190 and 280 grams and were 2 to 3 months old at the time of surgery. Animals were re-weighed on sacrifice dates. Only females were used, thus eliminating any sex-dependent response to surgery, wound healing, or infection.

B. Bacteria:

The bacteria were separate pure cultures of Staphyloccoccus aureus and Escherichia coli provided by Dr. J. Brunton of the Medical Bacteriology Department of the University of Alberta Hospital. These were obtained from two patients in the University of Alberta Hospital. One patient had a Staphylococcal septicemia and the bacterium was retrieved from a blood culture. The other patient had a sternal wound infection and the E. coli were retrieved by swab and culture techniques.

The two bacteria were sequentially plated on tryptone blood agar base with yeast extract less blood to ensure the presence of a single bacterial strain without contamination. The growth curves and innoculation quantity were determined (see methods) with the bacteria growing in tryptone soya broth. The innoculum was cryopreserved in 10% glycerol solution by cooling to -20°C overnight and then storing at -50°C. The viability of the bacterium was ascertained by duplication of growth kinetics and colony appearance.

C. Experimental Design

Three hundred and sixty animals were divided into three groups: controls, E. coli contaminated, and Staph. aureus contaminated. Each group was subdivided for primary or secondary closure. To study both the acute and chronic phases of infected wound healing, ten animals from each subgroup were sacrificed at 7, 14, 21, 42, 84 and 119 days after operation. If an animal suffered an immediate anaesthetic death, it was replaced but no attempt was made to replace animals that died after 24 hours. The two subgroups with the same contaminant and one of the control subgroups were operated on one day and the remaining three subgroups were operated on the following day. Selection for sacrifice corresponded to order of operation. Control groups were always operated upon first and separated from the infected subgroups post-operatively.

D. Operative Technique & Sample Collection

The rats were anaesthetized with 60 mg/kg of intraperiton phenobarbital. Th animals were weighed prior to surgery. The ventral abdominal wall was cleared of hair with an Oster animal clipper. No skin preparation was performed. The skin was sharply incised with a #11 scalpel blade and linea alba was identified. This was incised along with peritoneum for a length of 5 cm. with care taken not to traumatize the viscera. Hemostasis was achieved with gauze pressure. Peritoneum and rectus muscle and fascia were immediately closed with a single layer of interrupted simple sutures of 4-0 polyproplylene. The sutures were placed approximately 2-3 mm from the

wound edge and 7-9 mm apart and were tied with 3 throws. Once the abdominal wall was closed, bilateral subcutaneous pockets were bluntly dissected to the anterior axillary line. This was necessary to accommodate 1 ml of fluid. Pockets were dissected in all animals. One ml of sterile nutrient broth (see "Bacteriology") was placed in the control animals and 1 ml of the appropriate bacterial broth in the infected groups.

In those animals allocated to the primary closure subgroups, the skin was approximated with a simple continuous suture of 4-0 Prolene. Animals in the secondary closure subgroups were placed in a supine position after innoculation while still anaesthetized and observed to maintain this position for at least one hour.

When all three subgroups for a day were completed, they were returned to their individual cages in the vivarium.

At the time of sacrifice the animals were killed with 120 mg/kg of intrahepatic phenobarbital. A flap of skin approximately 4 x 7 cm was dissected free of the abdominal wall. In the secondary closure subgroups, where the skin edges had not come together by secondary intent, the overlying wound granulation tissue was left intact and the skin was removed up to the margin of granulation tissue. A 4 x 7 cm section of abdominal wall was then sharply elevated and any intraperitoneal adhesions were lysed. All of the sutures were removed by careful sharp dissection.

A 1 cm transverse strip of abdominal wall was collected for measurement of wound breaking strength. This strip never included an obvious hernia and was usually taken from the proximal or distal third

of the wound. These specimens were immediately wrapped in Parafilm and placed in a rubber stoppered glass vial.

A 1 x ? cm cross section of the midportion of the wound was submitted for histology. Obvious hernia sites were avoided. The specimen was promptly fixed in Bouin's solution. A 10 x 1 mm cross-section of the wound was promptly fixed in glutaraldehyde for scanning electron microscopy. Similar sampling precautions were observed.

Samples for hydroxyproline assay were harvested from the wound tissue without adjacent rectus fascia and muscle. It should be noted that as the wound matured and remodelled, wound sampling became more difficult because of the paucity of tissue. These specimens were placed in glass vials with rubber stoppers and rapidly cooled to -20°C in the cryostat.

E. Bacteriology

Staph. aureus were isolated from blood culture and identified by the Department of Medical Bacteriology at the University of Alberta Hospital. Stock cultures on blood agar plates were refrigerated at -4°C prior to transfer. Upon receipt, a single colony was streaked onto the nutrient agar, tryptone blood agar base with yeast extract. After a 24 hour incubation period at 37°C, inspection revealed a uniform colony appearance, indicating absence of other bacteria. The strain of E. coli isolated from the wound culture was also obtained from the UAH Microbiology Department and was stocked on egg slants. Upon receipt, E. coli from a single colony were streaked on the same nutrient agar and gave a uniform appearance at 24 hours.

The respective bacteria were then assayed for growth characteristics. Single colony samples were incubated in a nutrient broth, Oxoid in tryptone soya broth, overnight at 37°C. The resulting turbid broths were then sampled. Optical densities were measured in Canlab 45 mm semi-micro curvettes with the Unicam SP 1800 ultraviolet spectophotometer using a wavelength of 600 nm. Aliquots were added to sterile broth so to give an E. coli culture with an optical density of 0.07 and a Staph. aureus culture with an optical density of 0.045. The bacterial cultures were then placed in a 37°C water bath and samples for optical density and colony counts were withdrawn every 30 minutes for 3 1/2 hours. The optical densities were immediately recorded and the changes with time plotted (Fig. 1,2).

The samples for colony counts were withdrawn and immediately placed on ice. The samples were then serially diluted (10 fold) and appropriate dilutions were plated on the nutrient agar. The agar plates were incubated overnight at 37°C and colony counts were performed the following morning. Colony counts were multiplied by the dilution factor to give the bacterial concentration. The change in bacterial concentration with time is shown in figures 3 and 4 and the relationship with optical density in figures 5 and 6.

Pilot studies on the operative model with primary closure were performed using 10^7, 10^8, 5×10^8 and 10^9 organisms. No evidence of infection was found in animals contaminated with 10^7 and 10^8 E. coli or Staph. aureus. Using 10^9 Staph. aureus, two of four rats suffered massive abdominal wall necrosis and death. With a similar number of E. coli, one of four rats expired in the same manner. By

FIGURE 1. A calibration curve to relate Staph. aureus optical density at 600 nm. with time

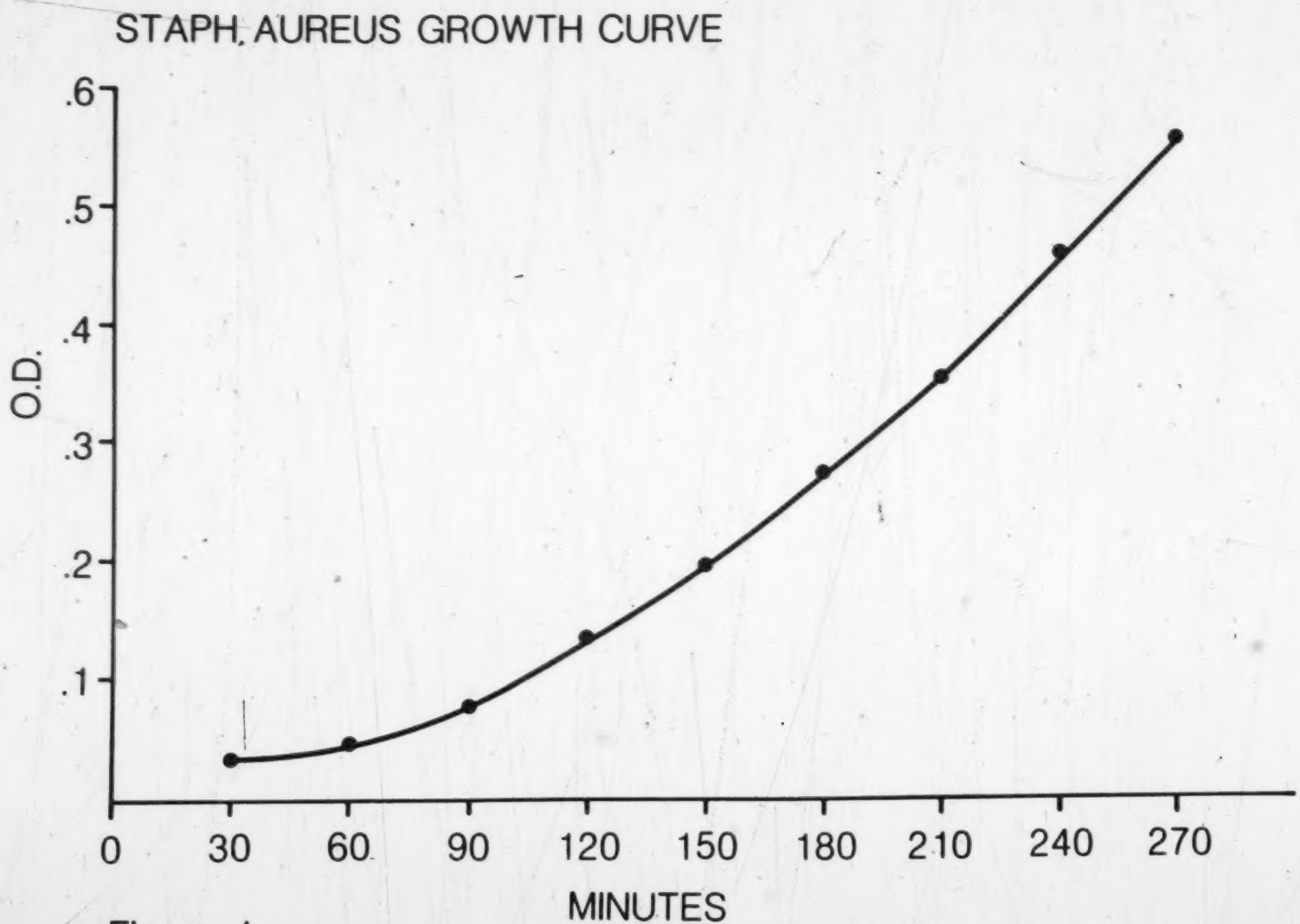

STAPH. AUREUS GROWTH CURVE
.6
.5
.4
.3
.2
.1
O.D.
0
30
60
90
120
150
180
210
240
270
MINUTES

Figure 1

FIGURE 2. A calibration curve to relate E. coli optical density at 600 nm. with time

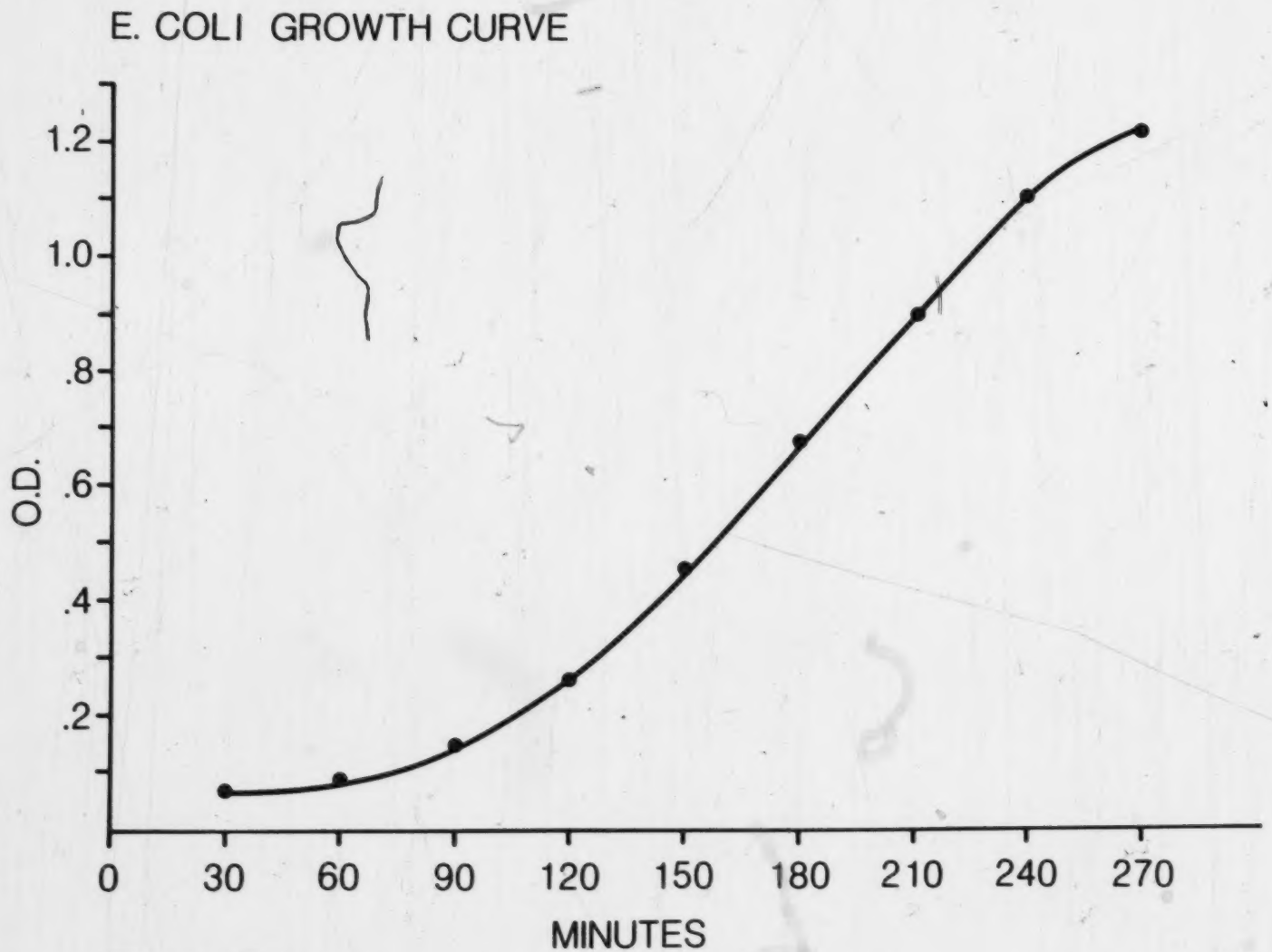

E. COLI GROWTH CURVE
O.D.
1.2
1.0
.8
.6
.4
.2
0
30
60
90
120
150
180
210
240
270
MINUTES

Figure 2

FIGURE 3. A calibration curve to relate Staph. aureus replication with time

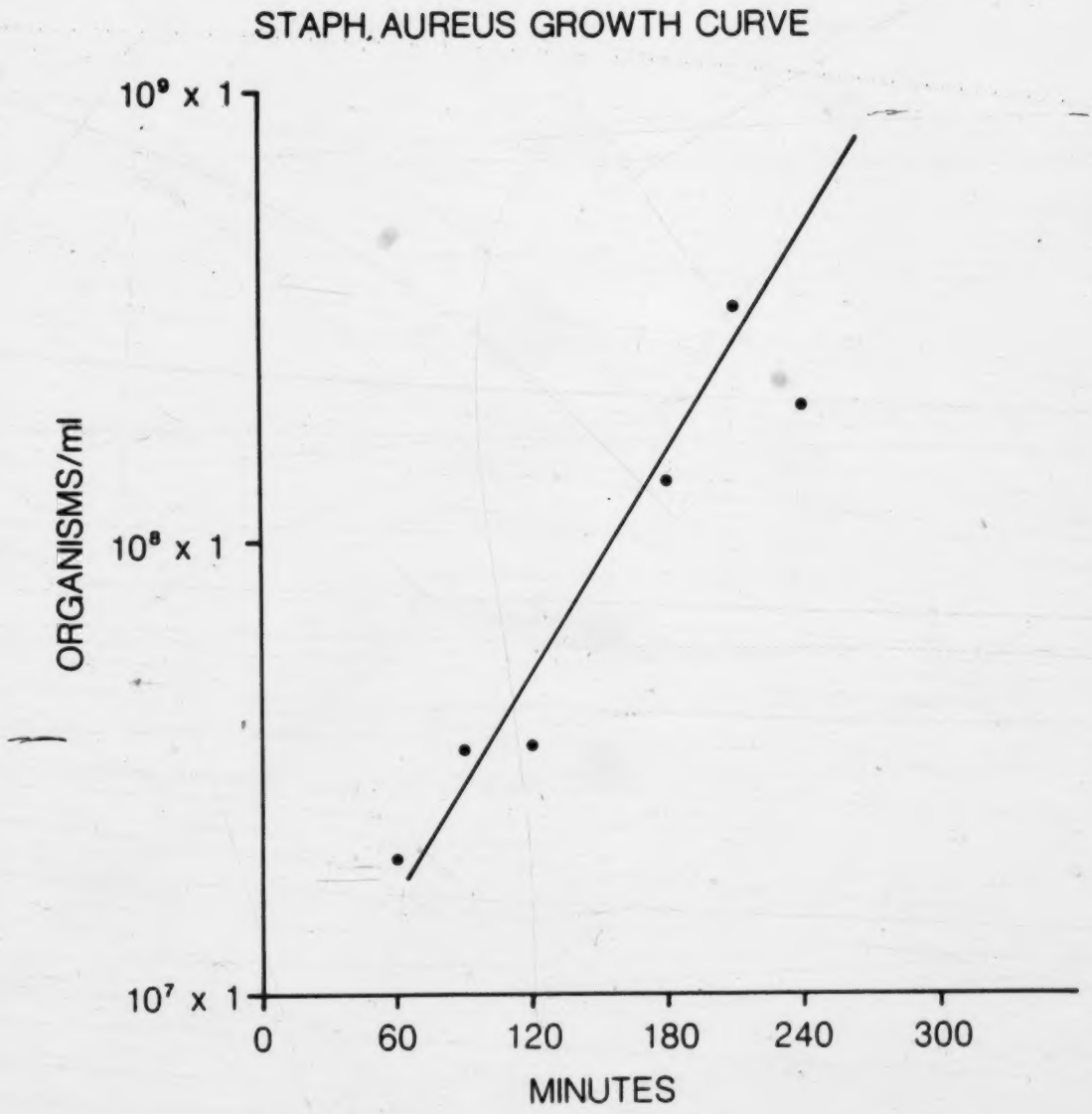

STAPH. AUREUS GROWTH CURVE
10^9 x 1
10^8 x 1
10^7 x 1
ORGANISMS/ml
0
60
120
180
240
300
MINUTES

Figure 3

FIGURE 4. A calibration curve to relate E. coli replication with time

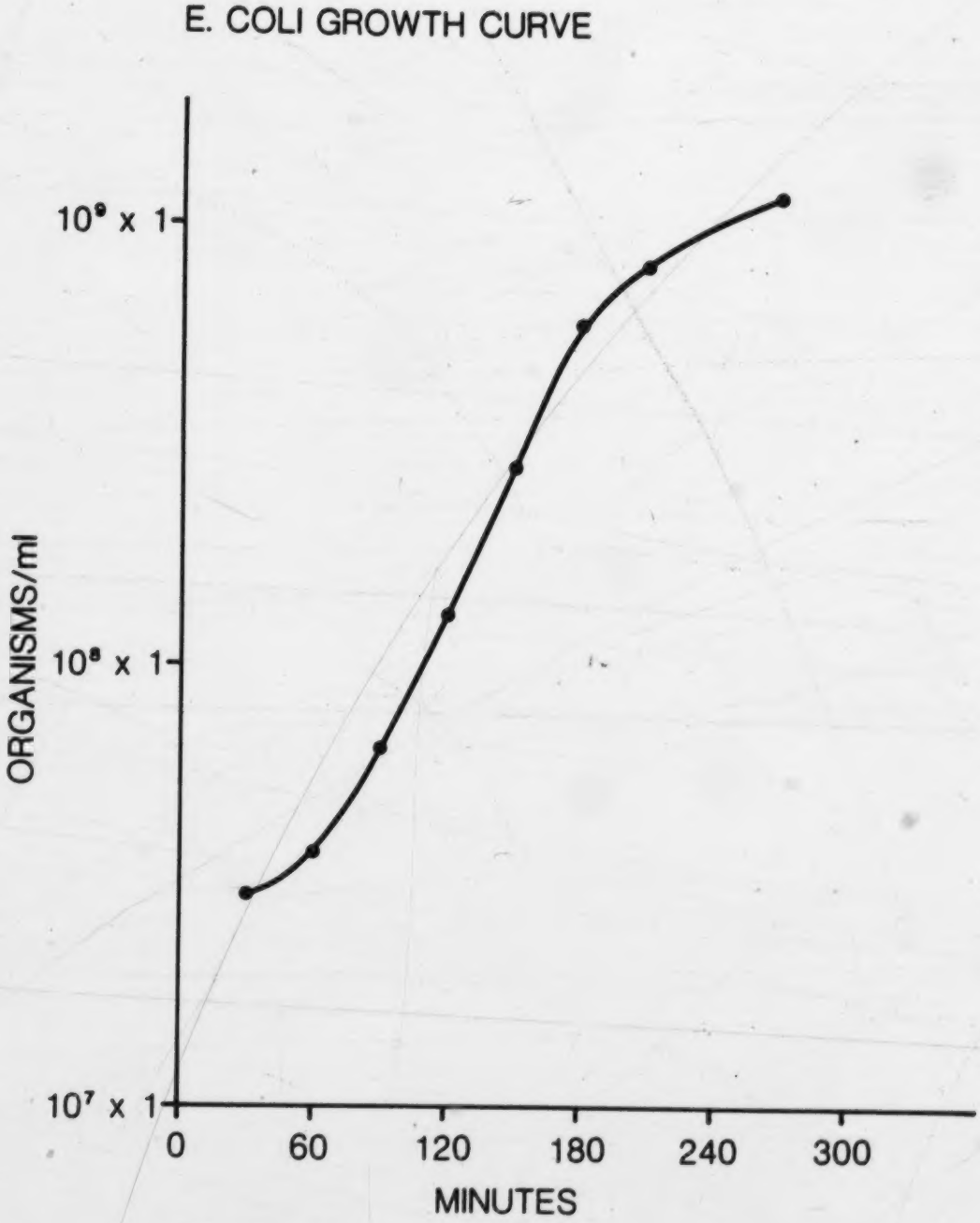

E. COLI GROWTH CURVE
ORGANISMS/ml
10⁹ x 1
10⁸ x 1
10⁷ x 1
0
60
120
180
240
300
MINUTES

Figure 4

FIGURE 5. A calibration curve to relate Staph. aureus concentration with optical density measured at a wavelength of 600 nm.

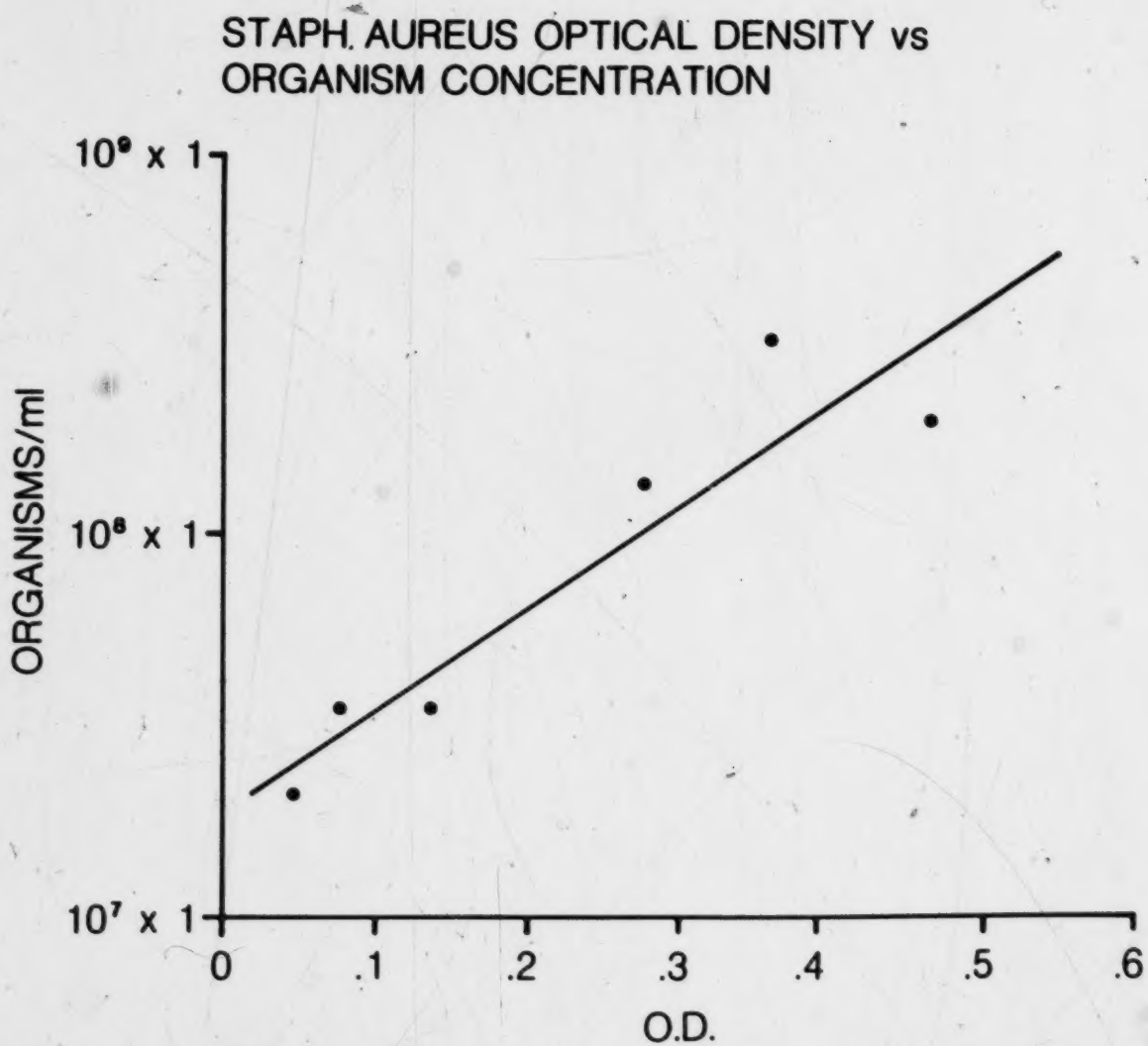

STAPH. AUREUS OPTICAL DENSITY vs
ORGANISM CONCENTRATION
ORGANISMS/ml
10⁹ x 1
10⁸ x 1
10⁷ x 1
0
.1
.2
.3
.4
.5
.6
O.D.

Figure 5

FIGURE 6. A calibration curve to relate E. coli concentration with optical density measured at a wavelength of 600 nm.

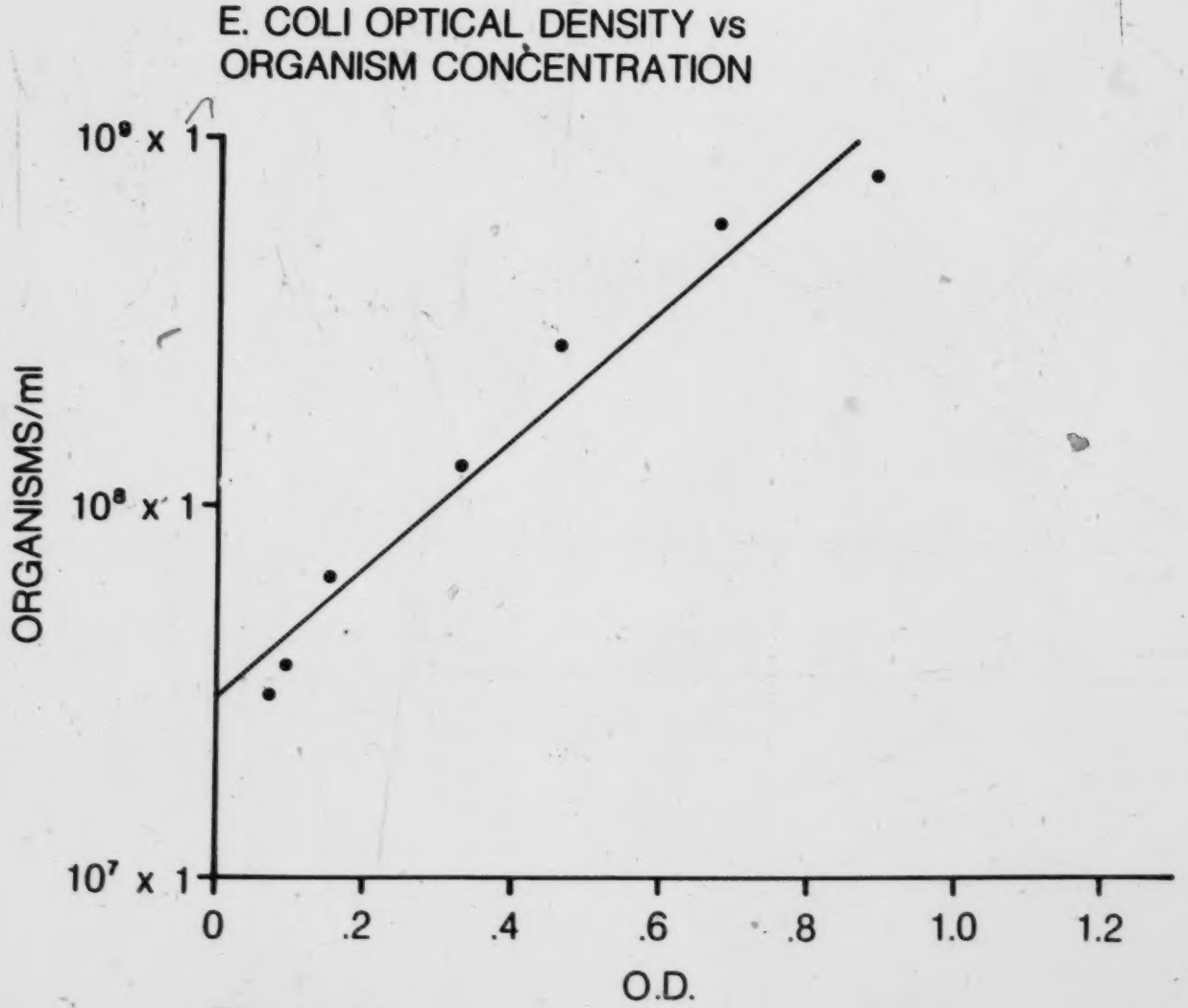

E. COLI OPTICAL DENSITY vs
ORGANISM CONCENTRATION
ORGANISMS/ml
10⁹ x 1
10⁸ x 1
10⁷ x 1
0
.2
.4
.6
.8
1.0
1.2
O.D.

Figure 6

contaminating the wounds with 5 x 10^8 Staph. aureus, two of three animals suffered wound infections without any evidence of associated morbidity or mortality. With the same number of E. coli, two of three animals responded similarly. To avoid excessive animal mortality and suffering, the minimum number of bacteria (5 x 10^8 organisms/ml) necessary to produce infection was used(53).

The bacteria were regrown under the same conditions to the optical density corresponding to 5 x 10^8 organisms/ml and placed on ice. Twenty ml aliquots of bacterial broth were thoroughly mixed with 2.2 ml of glycerol in universal bottles to give a 10% glycerol solution. This solution was promptly cooled to -20°C overnight and then placed in a -50°C refrigerator until used.

The viability of the organisms after cryopreservation was proven by duplication of growth curves after thawing and similar incidence of inflammation in pilot studies.

At the time of innoculation, the solution was brought to room temperature in a water broth. The bacteria suspensions were added to the wounds.

F. Scanning Electron Microscopy(54)

The tissue selected for S.E.M. was sharply dissected from the abdominal wall flap. The tissue was deliberately narrow (1 mm) to allow for more thorough fixative penetration and short (1 cm) to avoid curling. Since S.E.M. studies were primarily directed at collagen morphology and not cellular detail, perfusion techniques were not required.

After sampling, the tissue was directly placed in a sealed vial of 2.5% glutaraldehyde prepared in Millonig's buffer (16.8 g $NaH_2PO_4H_2O$, 3.8556 g NaOH, 5.4 g. glucose and .05 $CaCl_2$ in 1 liter of distilled water). Glutaraldehyde fixation was carried out for at least 24 hours after which the glutaraldehyde was removed by washing with buffer. The tissue was then post-fixed in 10% osmium tetroxide (prepared in Millonig's buffer) for 4 hours. The tissue was then washed in distilled water to remove the osmium tetroxide. The fixed tissue specimens were dehydrated through a graded series of aqueous alcohol solutions (50, 70, 80, 90, 95% and absolute alcohol) for a period of 15 minutes per solution. The absolute alcohol wash was repeated twice and the tissue was transported to the SEM laboratory in the third wash. No intermediate fluid was used. The tissue was further dried by CO_2 critical point drying and then coated with gold-palladium alloy.

The Cambridge 5150 scanning electron microscope was used and particular attention was directed at the wound collagen and appropriate photomicrographs were taken. It should be noted that only one specimen from each subgroup at each sacrifice date with the breaking strength closest for that subgroup was processed beyond glutaraldehyde fixation.

G. Tensile Strength Testing[55,56,57]

The 1 cm strips of abdominal wall were wrapped in parafilm and transported in rubber stoppered glass vials. The time between harvesting and testing was usually 4 hours, however, on two occasions

the testing apparatus malfunctioned and the specimens were stored at -50°C overnight. Breaking strengths prior to and after freezing showed no significant differences.

The Instron Universal Testing Instrument, metric table model TM-M, (Instron,Canton, Mass.) was used by courtesy of the Faculty of Home Economics at the University of Alberta. A CM Tension cell was used allowing for calibration through the range of breaking strengths of the tissue. An applied load on the cell caused a proportional change in the resistance of the strain gauges, which are arranged in a bridge circuit and excited by a stabilizer oscillator. The resulting signal is amplified and calibrated, and finally rectified to operate the recording pen. A synchronous motor drives the moving crosshead at a constant rate while the tension in the tested material is monitored by the load cell.

Clamps designed to grip the tissue were specially constructed in the Mechanical Workshop, Faculty of Medicine. These were held in place by the hydraulic jaws of the Instron loading frame. The tissue clamps had a 0.5 mm deep pattern of diagonal grooves and were opposed by two simple screws at each end. This system provided a firm purchase on the tissue without crushing and minimal slippage or shredding upon extension. The clamps were applied 2-3 mm from the wound edge so that wound, and not neighbouring muscle and fascia, would be tested. If breakage did not occur at the wound, the sample was retested. The clamps were separated at a rate of 20 cm/minute.

H. Histology

The specimens for histological examination were promptly fixed in Bouin's solution (75ml of saturated aqueous picric acid solution, 25 ml of 37-40% formaldehyde, and 5 ml of Glacial acetic acid).

The specimens were prepared at the Charles Camsell Hospital. The tissues first underwent commercial automated processing which completes the fixation, dehydrates in alcohol, "clears" in toluene and performs the paraffin wax embedding. Standard haematoxylin and eosin stains[58], Gomori's one-step trichrome stains[59], and Gordon and Sweet's reticulin impregnation stains[58] were performed on paraffin sections. Haematoxylin and eosin stains were used to assess the leukocyte infiltration, inflammation changes and maturation of the granulation tissue. The Gomori stain was used to assess relative collagen content and organization and the Gordon and Sweet stain was used to assess the same characters of reticulin fibres. Dr. P. Petrik of the Edmonton General hospital read the slides and scored his results on a check sheet. The slides were re-labelled to avoid influencing his interpretations.

I. Biochemistry Methods

Hydroxproline Determination[60,61]

Tissue specimens were lyophylized and then weighed. There was a large range (1.32 - 57.31 mg) of weights as the amount of granulation tissue changed considerably with time. Ten ml of 6N HCl was added to the sample and the sample was autoclaved for 3 hours at 125°C. One ml of sample was dried on the Buchler Evapo-mix and 2 ml of distilled

water was added. A sample (0.5 ml) was added to 0.5 ml of distilled water and mixed with 1 ml of isopropanol. Hydroxyproline was determined colorimetrically by the method of Bergman and Loxley[60]. A 7% w./v. aqueous solution of Chloramine T (0.5 ml) in an acetate/citrate buffer was mixed with the sample solution and four minutes later 1 ml of Ehrlich's reagent solution was admixed. This solution was placed in a water bath at 60°C for 25 minutes and put on ice for 60 minutes.

Spectrophotometry readings were then performed at 562 nm on the Cambridge Unicam SP1800 Ultraviolet Spectrophotometer. The optical density of the samples was compared to standard hydroxyproline solutions and the amount of hydroxyproline per unit weight of lyophilized tissue was calculated.

RESULTS

A. Wound Breaking Strength Measurements:

1. Controls

The wound breaking strength means, standard errors, standard deviations and coefficients of variance for both control subgroups are listed in table 1. The mean values with standard error of the mean of the primary and secondary closure samples are compared in figure 7.

The breaking strengths from day 7 to 21 in the primary closure subgroups show a relatively constant rapid gain from 0.36 kg tension to 0.99 kg tension. This is followed by a period with a more gradual gain to day 84 (1.69 kg) and a second, less pronounced increase to 2.10 kg at 119 days.

In the secondary closure subgroup the 7 to 21 days period also showed a rapid rise (although the mean strength at 14 days was relatively decreased). From day 21 to 119 there was a gradual continuous increase in breaking strength.

Comparison of the two control subgroups shows that secondary closure resulted in consistently stronger wounds. Student's *t* tests were done at each interval (see table 2, column 1) and these differences were statistically significant ($p<0.05$) on 4 of 6 sacrifice dates (days 7, 21, 84 and 119). The difference at 7 days may have been due to the granulation tissue present with secondary closure. It is noted that the breaking strength measurements of all subgroups at 7 days are similar and much less than those of the

TABLE 1

Values of Wound Breaking Strengths in (kg.) Primary and Secondary Closure in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Control Animals

Day	7		14		21		42		84		119	
Closure[a]	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°
Mean	0.46	0.73	0.67	0.77	0.99	1.38	1.21	1.52	1.69	2.48	2.10	2.40
S.D.	0.23	0.26	0.07	0.36	0.26	0.28	0.59	0.47	0.60	0.70	0.62	0.76
S.E.	0.08	0.09	0.02	0.15	0.09	0.09	0.21	0.15	0.18	0.35	0.21	0.25
Coeff.var.[b]	49.91	35.40	10.10	46.46	25.77	20.53	48.50	31.26	37.70	28.19	29.68	26.28

Infected with Staph. aureus

Day	7		14		21		42		84		119	
Closure	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°
Mean	0.45	0.29	0.74	0.03	1.30	1.27	1.54	1.13	2.53	2.42	2.60	2.87
S.D.	0.14	0.14	0.50	0.36	0.41	0.35	0.63	0.34	0.50	0.82	0.57	0.87
S.E.	0.05	0.06	0.17	0.11	0.14	0.11	0.21	0.13	0.16	0.26	0.22	0.24
Coeff.var.	31.36	46.46	66.91	43.40	31.90	27.68	40.56	34.13	19.65	34.06	21.88	30.44

Infected with E. coli

Day	7		14		21		42		84		119	
Closure	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°
Mean	0.36	0.30	0.36	0.58	1.39	1.20	1.37	1.48	1.17	2.45	1.85	2.30
S.D.	0.17	0.30	0.12	0.27	0.54	0.27	0.56	0.57	0.38	0.58	0.54	0.81
S.E.	0.07	0.09	0.04	0.10	0.17	0.09	0.18	0.18	0.12	0.18	0.17	0.26
Coeff.var.	44.99	48.70	32.05	31.15	38.62	22.59	41.23	38.75	32.62	23.67	28.42	35.07

[a] 1°, primary, 2°, secondary

[b] Coefficient of Variance

FIGURE 7. A comparison of primary (1°) and secondary closure (2°) wound breaking strengths in control rats. Standard error of the mean (S.E.M.) is represented by the bars.

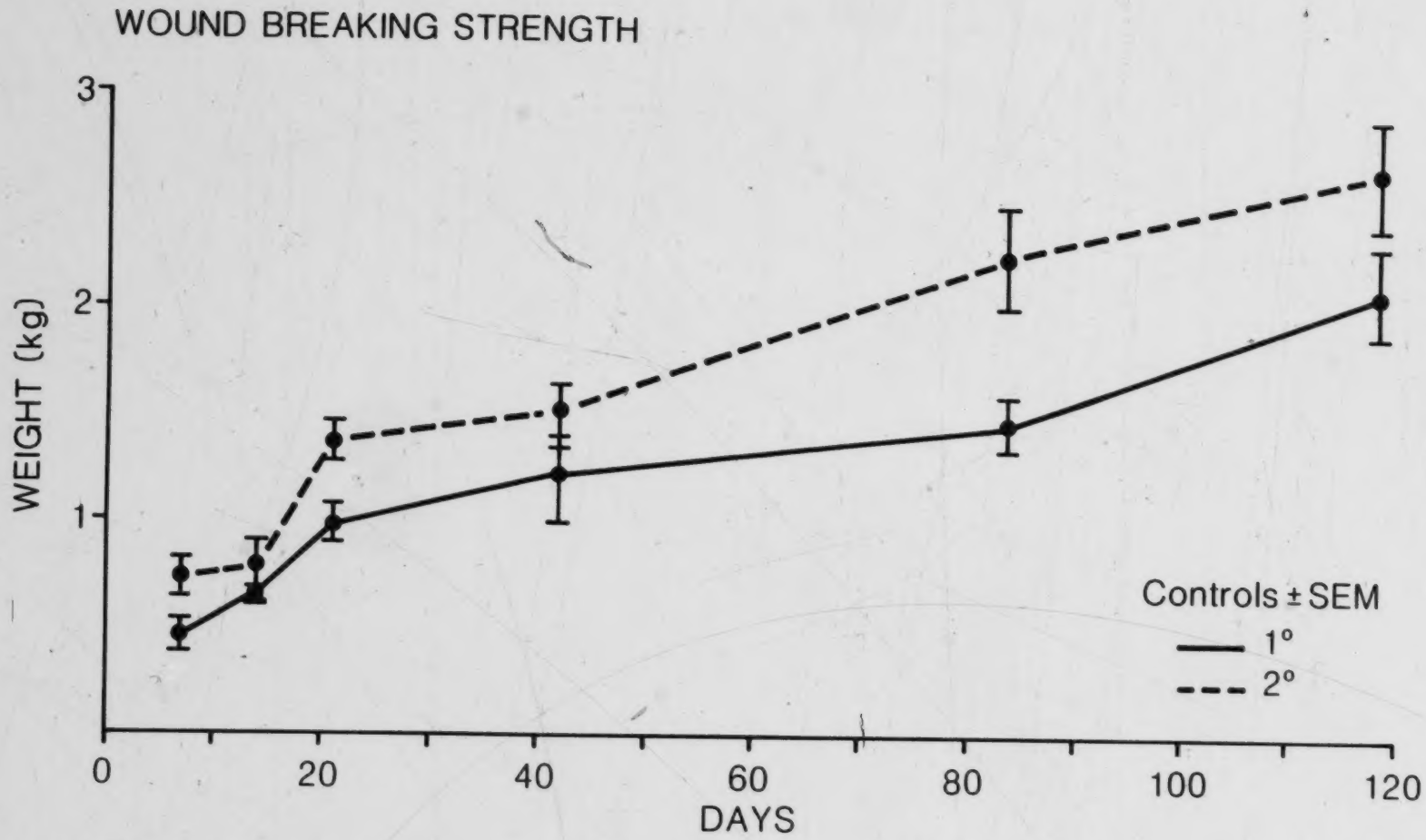

WOUND BREAKING STRENGTH
WEIGHT (kg)
3
2
1
0
20
40
60
80
100
120
DAYS
Controls ± SEM
1°
2°

Figure 7

TABLE 2

Statistical Evaluation (Student's *t* test p values) of Wound Breaking Strengths in Rats: A Comparison of Experimental with Primary Closure Control Wounds

	Non-infected	Staph. aureus Infected		E. coli infected	
	2°	1°[a]	2°[b]	1°	2°
Days					
7	†<0.05	†<0.6(n.s.)	†<0.01(n.s.)	†<0.9(n.s.)	†<0.4(n.s.)
14	†<0.5(n.s.)	†<0.7(n.s)	†<0.3(n.s.)	†<0.001	†<0.1(n.s.)
21	†<0.01	†<0.4(n.s.)	†<0.1(n.s.)	† 0.1(n.s.)	†<0.2(n.s.)
42	†<0.2(n.s.)	†<0.4(n.s.)	†<0.5(n.s.)	†<0.6(n.s.)	†<0.4(n.s.)
84	†<0.02	†<0.001	† 0.01	†< .2(n.s.)	†<0.001
120	†<0.05	†<0.2(n.s.)	† 0.05	†<0.5(n.s.)	†<0.9(n.s.)

[a] Primary closure

[b] Secondary closure

control secondary closure group. It was noted that the granulation tissue in both infected secondary closure subgroups appeared wet and friable in comparison to the firm granulation tissue in the control secondary closure subgroup. It is assumed that this feature was a major contributing factor to increased strength at this test time. This finding was not obvious at any other sacrifice date.

On the two other sacrifice dates, the control secondary closure subgroups' breaking strength measurement means were greater than those of primary closure but these differences were not statistically significant. At day 14 the p value was 0.5 and on day 42, the p value was 0.2.

2. Staphylococcus Infected Animals

Examination of the wound breaking strength measurements in the Staph. aureus infected animals (Fig. 8) shows comparable results between primary and secondary closure subgroup. Only at 7 days was there a significant difference with the primary closure subgroup being stronger ($p<0.02$). The values obtained are consistent with the normal pattern of healing with a rapid rise during the first three weeks and then a gradual gain during the remainder of the test period.

There are several noteworthy differences between Staph. aureus infected animals and controls. Other than the difference noted at 7 days, comparison of the two Staph. aureus infected subgroups (Fig. 9) with the primary closure control subgroup shows minimal differences from days 7 - 42 (for p values see Table 2, columns 3 & 4). However, at 84 days both the primary and secondary closure Staph infected-wounds (Table 2)

FIGURE 8. A comparison of primary (1°) and secondary (2°) closure wound breaking strengths in rats infected with Staph. aureus. Standard error of the mean (S.E.M.) is represented by bar graphics.

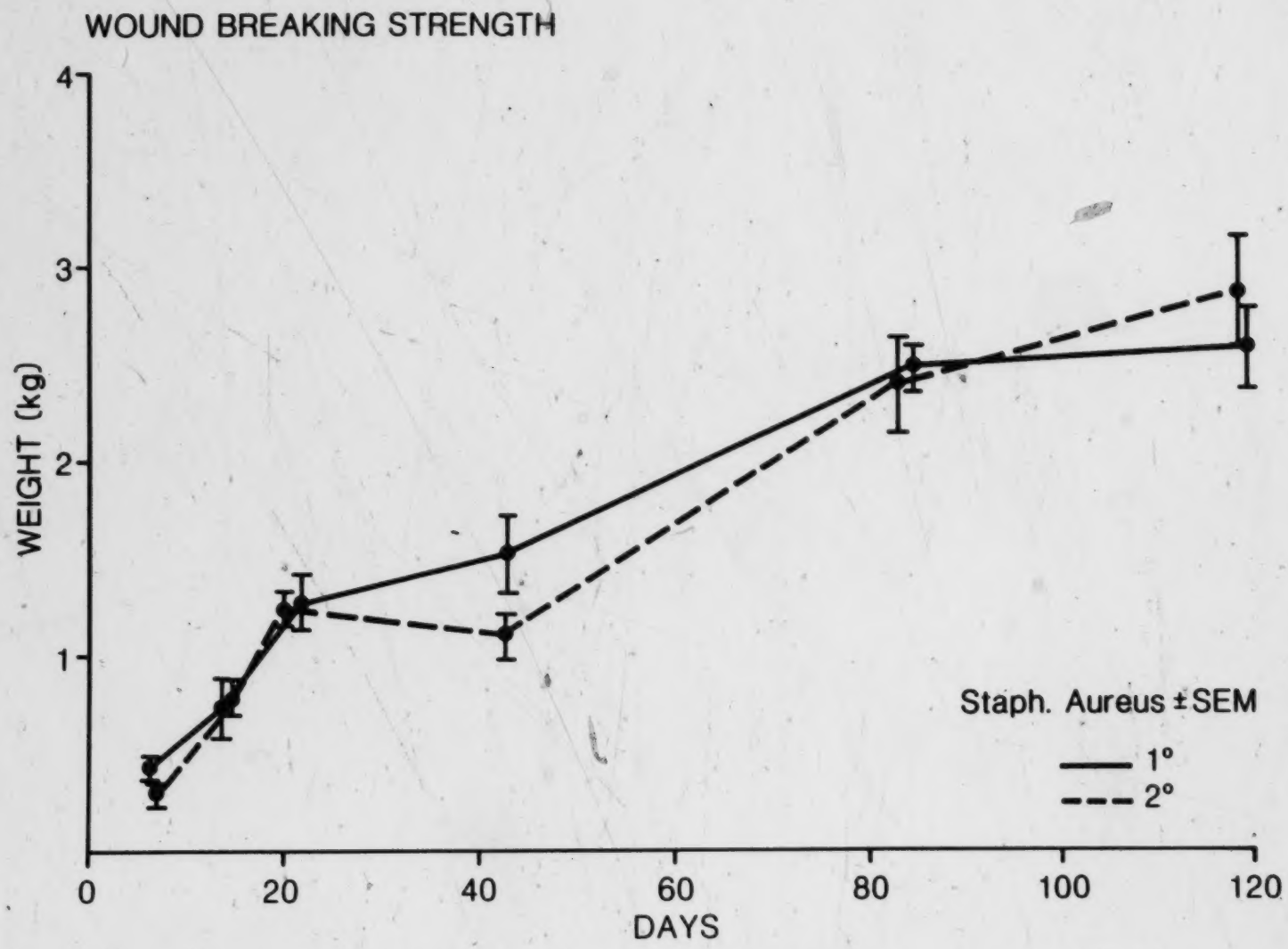

WOUND BREAKING STRENGTH
WEIGHT (kg)
4
3
2
1
0
20
40
60
80
100
120
DAYS
Staph. Aureus ± SEM
1°
2°

Figure 8

FIGURE 9. A comparison of control primary (1°) and secondary (2°) closure wound breaking strengths with primary (1°) and secondary (2°) closure wounds infected with Staph. aureus. Standard error of the mean (S.E.M.) is represented by bar graphics.

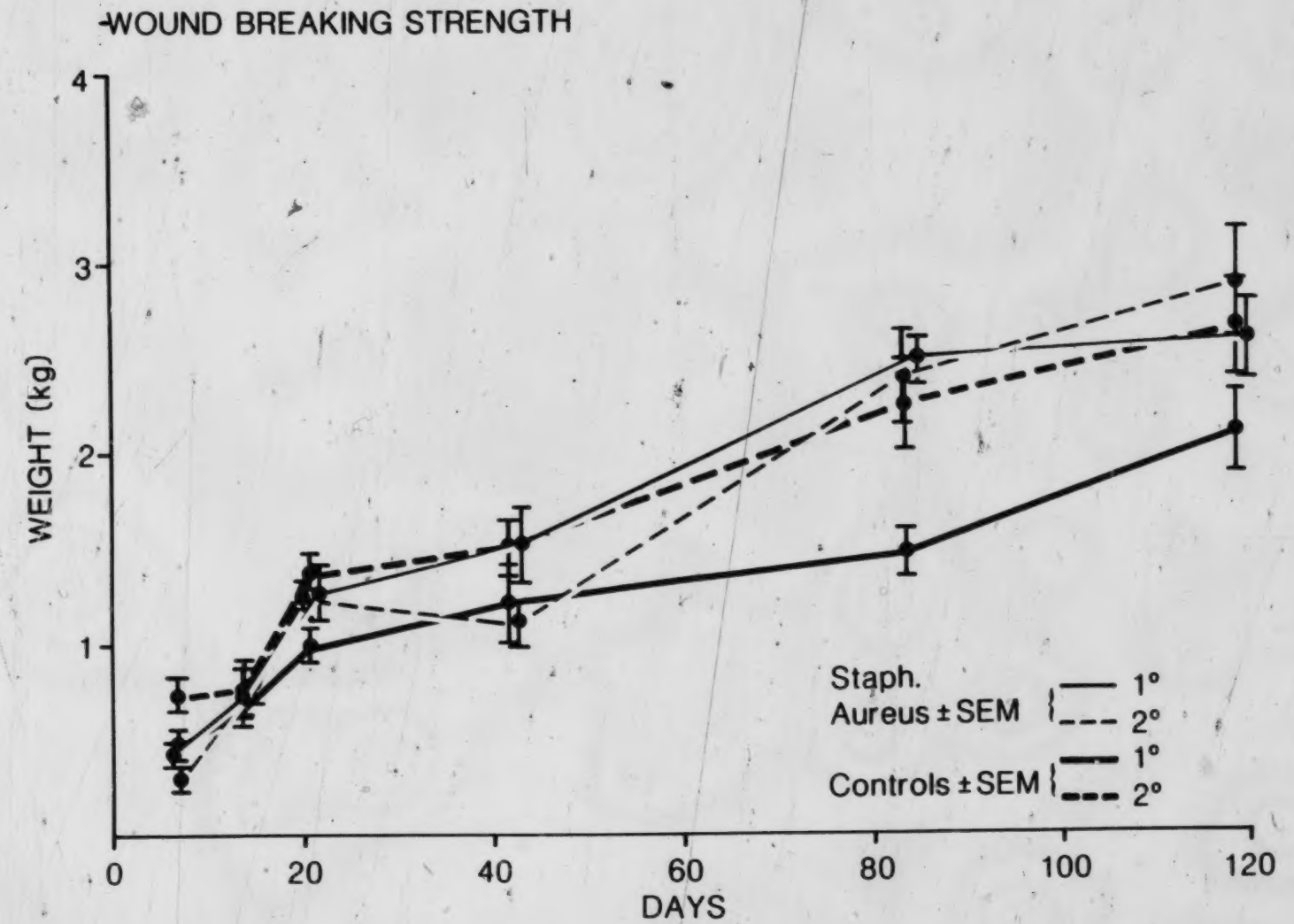

WOUND BREAKING STRENGTH
WEIGHT (kg)
4
3
2
1
0
20
40
60
80
100
120
DAYS
Staph. Aureus ± SEM
1°
2°
Controls ± SEM
1°
2°

Figure 9

TABLE 3

Statistical Evaluation (Student's *t* Test p Values) of Differences In Wound Breaking Strengths in Rats: A Comparison of Primary and Secondary Infected Wound Closures with Secondary Control Wound Closure

	Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°
Days				
7	$t<0.02$	$t<0.001$	$t<0.05$	$t<0.01$
14	$t< .5$ (n.s.)	$t< .8$ (n.s.)	$t< .02$	$t< .6$ (n.s.)
21	$t< .7$ (n.s.)	$t< .5$ (n.s.)	n.s.	$t< .4$ (n.s.)
42	$t< .4$ (n.s.)	t 0.05	$t< .2$ (n.s.)	$t<0.1$ (n.s.)
84	$t< .4$ (n.s.)	$t< .4$ (n.s.)	$t<0.001$	$t< .4$ (n.s.)
120	$t< .4$ (n.s.)	- (n.s.)	t 0.01	t .2 (n.s.)

[a] Primary Closure

[b] Secondary Closure

are stronger than the primary closure control wounds ($p<0.01$ and $p<0.001$, Table 2), however, this difference becomes less apparent at 119 days as the primary closure control wounds showed a relatively greater gain in strength. Analysis proved that only the secondary closure Staph. aureus infected wound was statistically stronger ($p<0.05$) at this time.

A comparison of the Staph. aureus infected subgroups with the secondary closure control wounds (Fig. 9, & Table 3, column 1,2) shows comparable results for the most part, although there are two times at which significant differences are apparent. At 7 days the secondary closure control wound is stronger than both the primary closure Staph aureus infected subgroup ($p<0.02$, Table 3) and secondary closure infected subgroup ($p<0.001$, Table 3). As discussed previously, the firm granulation tissue in the secondary closure control subgroup was probably responsible for this difference. At 42 days, the secondary closure Staph. aureus infected subgroup was weaker ($p<0.05$) than the secondary closure control subgroup.

3. E Coli Infected Animals

A comparison of primary and secondary closure in the E. coli subgroups is shown in figure 10. The secondary closure subgroup shows changes with time that are similar to the previous group, i.e., a rapid gain in strength over the first three weeks, a gradual late gain, and a plateau phase. The primary closure subgroup is markedly different. Between day 7 and 14 there is a fall in strength which is followed by a compensatory rapid gain to levels comparable with those

FIGURE 10. A comparison of primary (1°) and secondary (2°) wound closure breaking strengths in rats infected with E. coli. Standard error of the mean (S.E.M.) is represented with bar graphics.

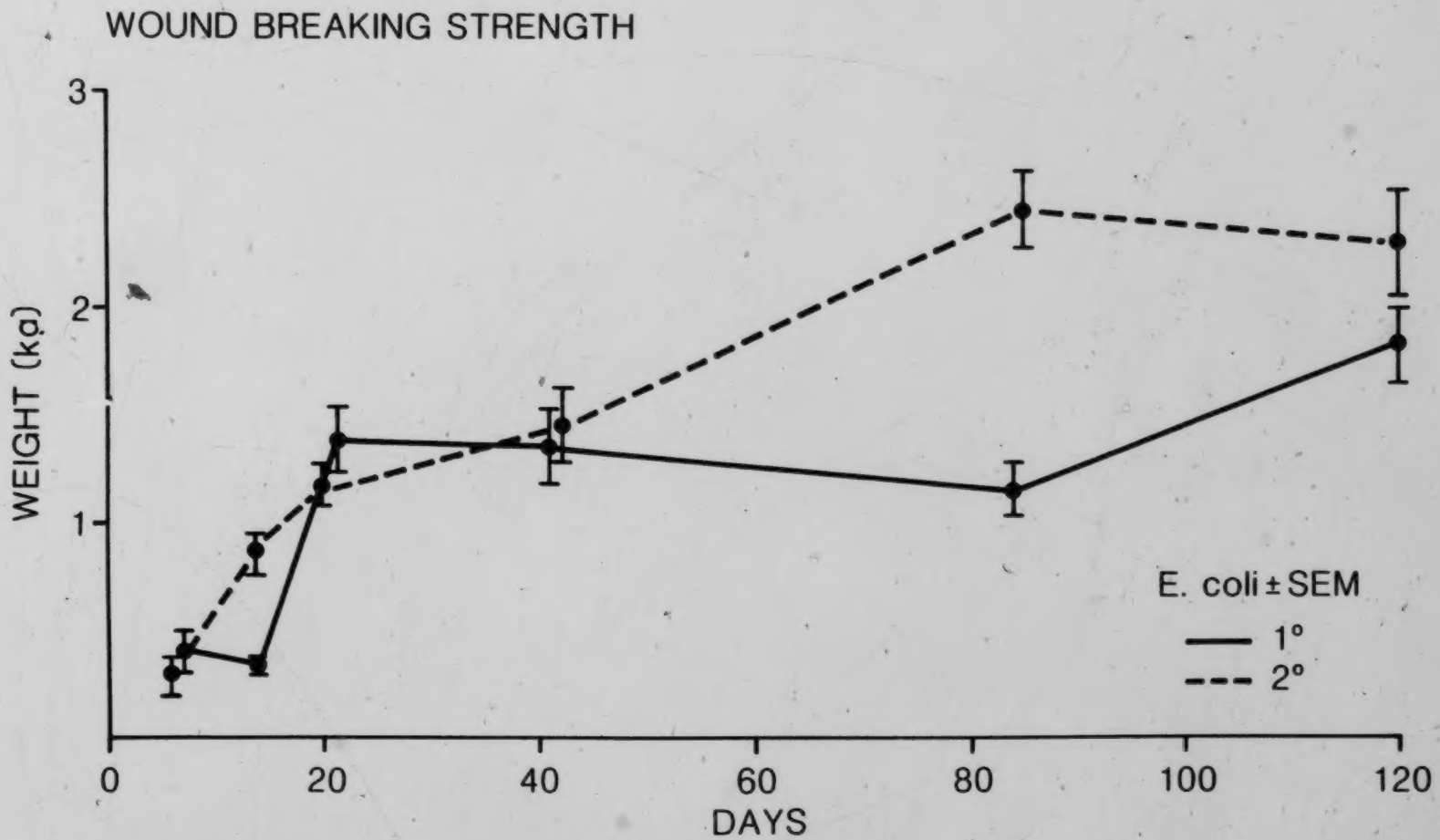

WOUND BREAKING STRENGTH
WEIGHT (kg)
3
2
1
0
20
40
60
80
100
120
DAYS
E. coli ± SEM
1°
2°

Figure 10

of controls at day 21. However, between day 21 and 84, there is a gradual decline in strength which was only found for this particular subgroup. There is a late gain in strength between days 84 and 119. These changes are significant when the stronger secondary closures are compared to the primary closures at day 14 ($p<0.01$) and at day 84 ($p<0.001$).

Relative to the primary closure control wounds (Fig. 11, Table 2 columns 4,5). the primary closure E. coli infected wounds were statistically weaker only at 14 days ($p<0.001$). The primary closure control wounds show a continual gain in strength throughout the test period, whereas the primary closure E. coli infected wounds lost strength between day 21 and 84.

The smooth continuous gain in the strength in the secondary closure control group can also be contrasted with the irregular pattern of changes in the primary closure E. coli infected wounds. The secondary closure control wounds are stronger than the primary closure infected wounds at all intervals other than day 21 when they are equal (Fig.10). These differences are statistically significant for days 7 ($p<0.05$), 14, ($p<0.02$, 84 ($p<0.001$) and 119 ($p<0.01$) (Table 3, column 4).

The values and patterns of change in the secondary closure control and secondary closure E. coli infected wounds are similar. There is a statistically significant difference only for day 7 when the control wounds were stronger ($p<0.01$) (Table 3, column 5). Again, this may be secondary to the firm granulation tissue.

FIGURE 11. A comparison of control primary (1°) and secondary (2°) closure control wound breaking strengths with primary (1°) and secondary (2°) closure wounds infected with E. coli. Standard error of the mean (S.E.M.) are represented by bar graphics.

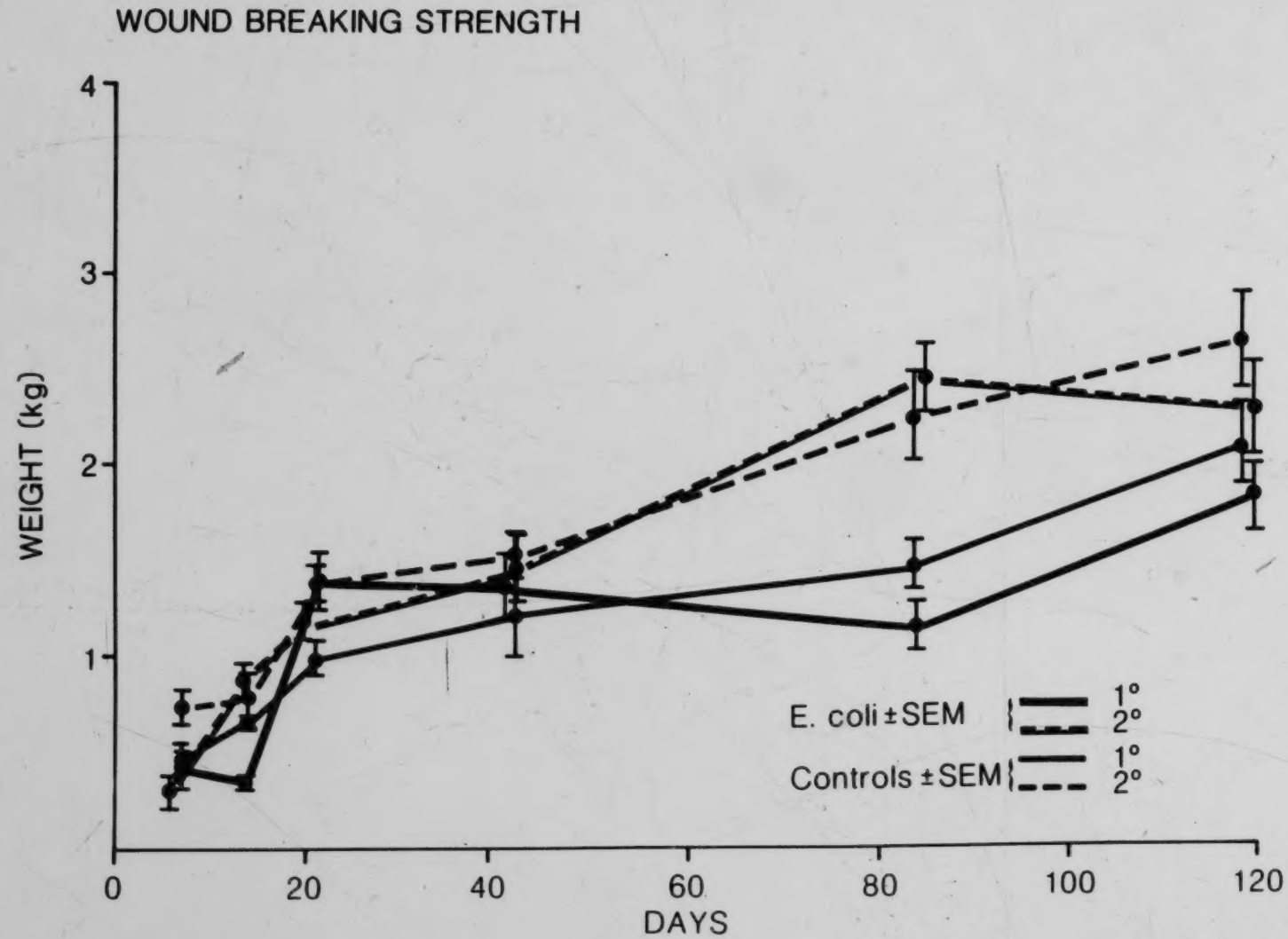

WOUND BREAKING STRENGTH
WEIGHT (kg)
4
3
2
1
0
20
40
60
80
100
120
DAYS
E. coli ± SEM
1°
2°
Controls ± SEM
1°
2°

Figure 11

Due to the design of the experiment, not all animals in the infected primary closure subgroups showed evidence of inflammation. Analysis of breaking strength measurement in these subgroups was repeated, excluding the non-inflamed wounds. In the Staph. aureus infected primary closure subgroup, this re-evaluation resulted in no significant changes of the student's t test p values between the primary infected closures and the primary control or the secondary infected closures. Therefore, in this case, exclusion of the non-inflammed wounds did not alter the results.

When this criterion was applied to the E. coli infected wounds, similar results were obtained except that the difference between the 84 day primary control and infected closure became significant ($p<0.05$).

B. Hydroxyproline Concentration of Tissue Samples

These are plotted in figures 12, 13, and 14. and the means, standard deviations, standard errors, and coefficients of variance are presented in table 4. A rather consistent pattern is apparent: the hydroxyproline content rises rapidly over the first three weeks, declines slightly, and then plateaus. The one exception to this is the E. coli infected secondary closure subgroup. There is a decreased hydroxyproline content at day 21 in relation to day 14. This implies either an earlier peak time or an absolute decrease at day 21 in this

FIGURE 12. A comparison of primary (1°) and sedondary (2°) closure wound hydroxyproline concentrations in control rats. Standard error of the mean (S.E.M.) is represented by bar graphics.

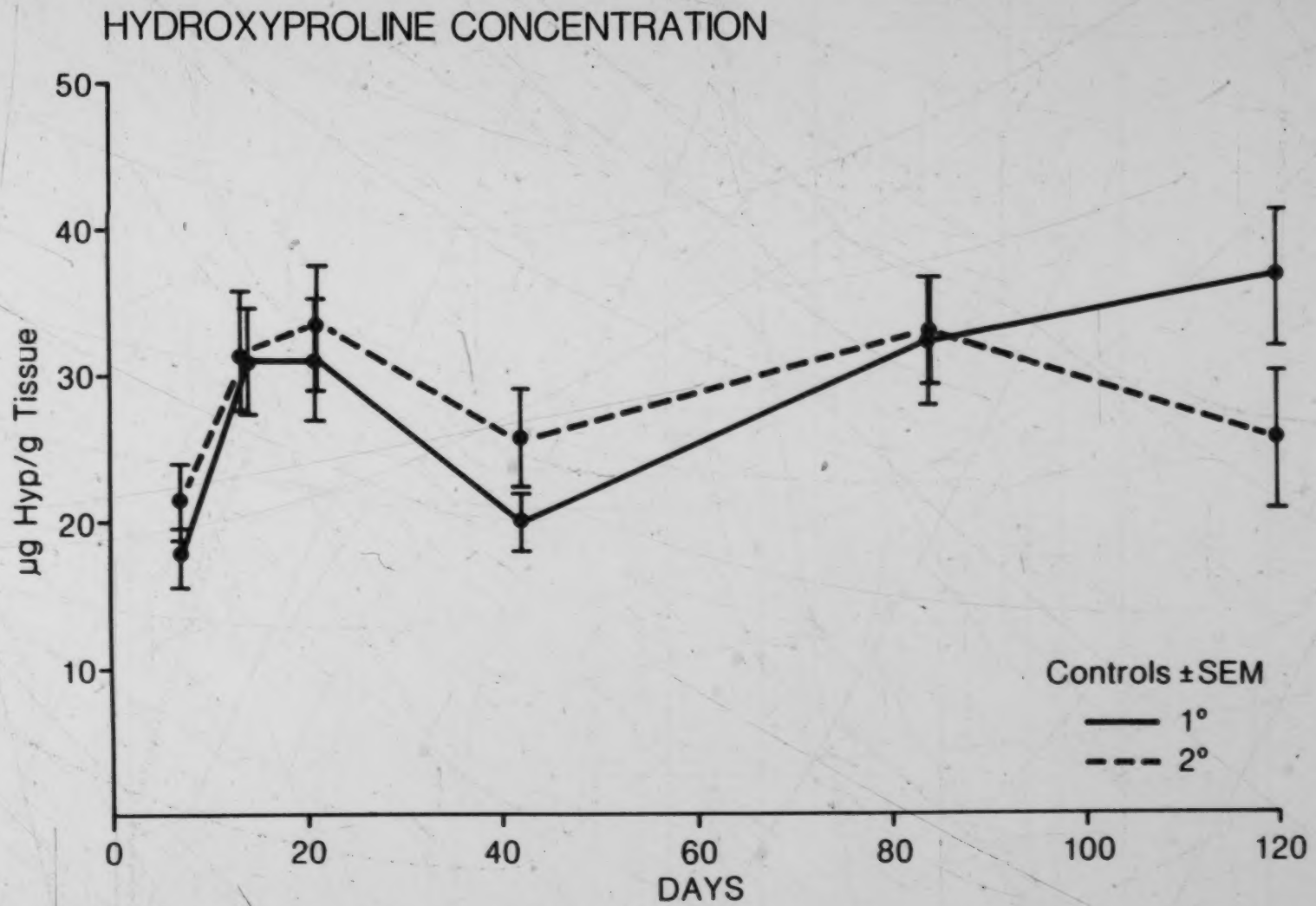

HYDROXYPROLINE CONCENTRATION
50
40
30
20
10
µg Hyp/g Tissue
0
20
40
60
80
100
120
DAYS
Controls ± SEM
1°
2°

Figure 12

FIGURE 13. A comparison of primary (1°) and secondary (2°) closure wound hydroxyproline concentrations in rats infected with Staph. aureus. Standard error of the mean (S.E.M.) is represented by bar graphics.

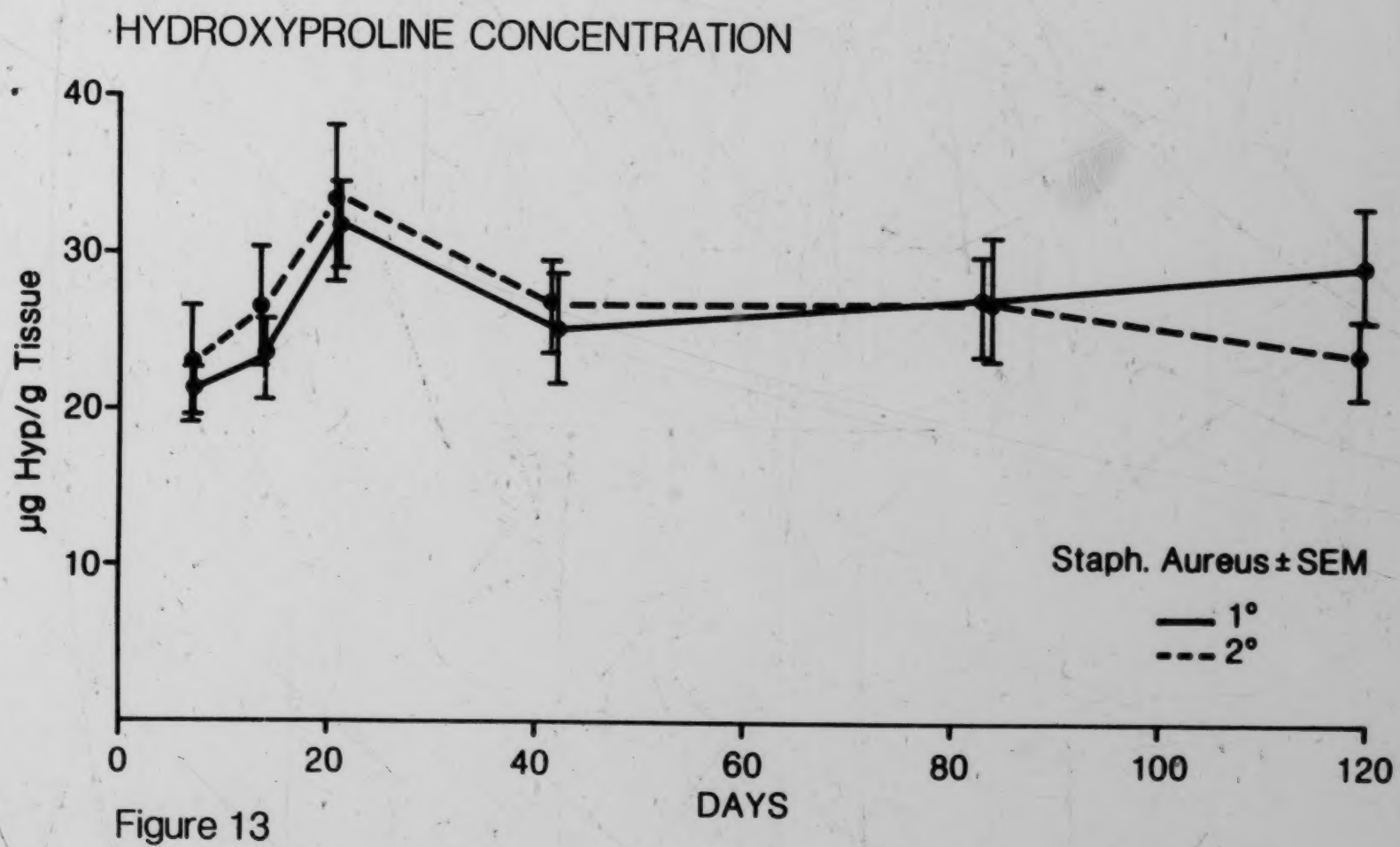

HYDROXYPROLINE CONCENTRATION
µg Hyp/g Tissue
40
30
20
10
0
20
40
60
80
100
120
DAYS
Staph. Aureus ± SEM
1°
2°

Figure 13

FIGURE 14. A comparison of primary (1°) and secondary (2°) closure wound hydroxyproline concentrations in rats infected with E. coli. Standard error of the mean (S.E.M.) is represented by bar graphics.

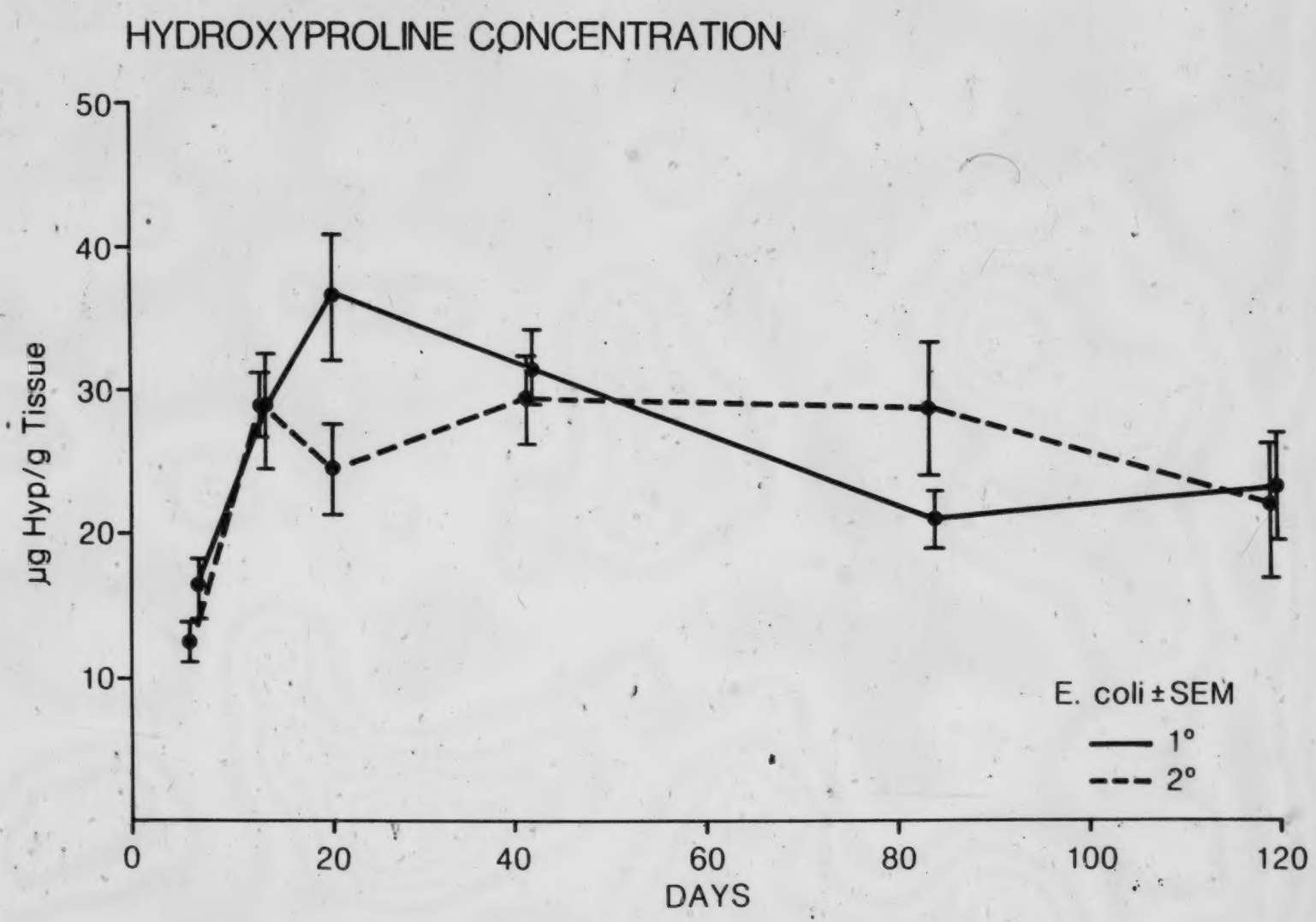

HYDROXYPROLINE CONCENTRATION
µg Hyp/g Tissue
50
40
30
20
10
0
20
40
60
80
100
120
DAYS
E. coli ± SEM
1°
2°

Figure 14

TABLE 4

Values of Hydroxyproline Concentration

(Micrograms of Hydroxyproline per gram of Dry Tissue Wound)

in Primary and Secondary Closure Wounds of Control, Staph. aureus-Infected, and C. coli-Infected Rats

Controls

Day	7		14		21		42		84		119	
Closure	I[a]	II[b]	I	II	I	II	I	II	I	II	I	II
Mean	17.56	21.11	30.71	31.93	30.99	33.45	20.11	25.83	33.17	32.08	36.63	25.42
S.D.	5.32	8.10	10.75	8.83	11.63	11.60	6.78	9.17	12.33	11.81	13.19	13.51
S.E.	1.88	2.56	3.40	3.60	3.74	3.67	2.26	2.90	3.72	3.7	4.40	4.56
Coeff.var.	36.29	38.39	35.0	27.63	38.17	34.67	33.7	35.50	37.18	33.7	36.0	53.13

Staph. aureus Infected

Day	7		14		21		42		84		119	
Closure	I[a]	II[b]	I	II	I	II	I	II	I	II	I	II
Mean	21.03	22.61	23.14	26.19	31.84	33.84	25.31	26.46	27.77	27.61	29.53	23.44
S.D.	4.44	8.46	7.87	10.76	7.46	13.12	9.65	8.35	10.70	9.72	11.0	7.46
S.E.	1.48	3.45	2.49	3.40	2.65	4.15	3.22	2.64	3.38	3.07	3.89	2.25
Coeff.var.	21.13	37.43	34.01	41.07	24.99	38.76	38.14	31.55	38.53	35.20	37.26	31.81

E. coli Infected

Day	7		14		21		42		84		119	
Closure	I[a]	II[b]	I	II	I	II	I	II	I	II	I	II
Mean	16.21	12.23	28.76	28.83	36.58	24.24	31.57	29.51	20.74	28.08	23.08	22.24
S.D.	5.92	3.60	10.0	6.17	13.23	9.66	7.29	9.35	5.47	13.37	10.54	12.85
S.E.	1.87	1.20	3.54	2.06	4.18	3.06	2.31	2.96	1.73	4.23	3.33	4.06
Coeff.var.	36.52	31.10	34.76	21.41	36.16	39.87	23.10	31.69	26.37	47.62	45.68	57.78

[a] Primary Closure

[b] Secondary Closure

subgroup. This pattern is consistent with the events occurring in the wound.

During the early (0-7 days) period of wound healing, the predominant features are those of hemostasis, inflammation and tissue debridement. Collagen synthesis is very low. This is followed by an intermediate (proliferative) phase, of rapid collagen synthesis. The wound then undergoes a final phase of remodelling, in which the balance between collagen synthesis and lysis results in a decreased total amount of collagen. It is apparent that these changes are reflected in the hydroxproline concentrations in figures 12, 13 and 14, thus validating the sampling technique.

In the control group, the values for the primary and secondary closures subgroups are similar. Differences at day 42 ($p<0.5$) and at day 119 ($p<0.1$) are not statistically different. This is in contrast to the wound breaking strength measurements which are significantly different at 4 of 6 sampling times.

In the Staph. aureus infected group, no statistically significant differences in hydroxyproline concentrations are apparent between primary and secondary closure subgroups. This parallels the pattern of wound breaking strength measurements. A comparison of the appropriate control and Staph. aureus infected subgroups shows only

one apparent difference in the 14 day primary closure sugroups which is not statistically significant ($p<0.1$).

In the E. coli infected group there is only one significant difference between subgroups which occurs at day 21 ($p<0.05$). However, there is no corresponding difference in wound breaking strength measurements at this time. A comparison of the appropriate control and E. coli infected subgroups, shows several differences in hydroxyproline concentrations . A day 7, the appropriate secondary closure control subgroup is statistically stronger ($p<0.01$) than the E. coli infected subgroup and this corresponds to a difference in strength. In the primaril closed subgroups, the E. coli infected wounds had a significantly greater ($p<0.01$) collagen concentration than control wounds at day 42 (strengths, however, were similar) and significantly less ($p<0.05$) collagen at day 119 (again, strengths were similar).

As noted previously, bacterial innoculation did not result in inflammation in all animals. When the hydroxyproline concentration measurements were excluded, no significant differences were found.

C. Animal Outcome

The course of the animals was monitored by examination of the wounds on the second and seventh post-operative days and at the time

of sacrifice. Four parameters (inflammation, abscesses, herniation and mortality) were studied.

The interpretation of the presence of inflammation is subjective. All wounds undergo an inflammatory phase in which the peri-wound tissues become edematous and erythematous. The criteria for describing a wound as inflamed were based on comparison with the control subgroups. The control animals were first examined to establish a standard for all subgroups of primary or secondary closure. Any excessive degree of erythema or edema or the presence of an exudate was taken to signify inflammation.

Abscesses were defined as fluctuant masses palpated in the operated area. At the time of sacrifice, any pocket of granulation tissue containing purulent material was also considered to be an abscess.

Herniae were defined as palpable defects of the incision or significant areas of membranous tissue at the wound site at sacrifice.

The incidence of inflammation is presented in table 5. A comparison of the primary closure subgroups shows that 1.75% of the control subgroup, 57.6% of the Staph. aureus infected subgroup and 56.6% of the E. coli infected subgroup had significant inflammation. Chi-square evaluation of this data revealed a highly significant difference ($p=1.68 \times 10^{-11}$) between control and primary closure infected subgroups. A comparison of the secondary closure subgroups shows that 7.27% of the control subgroup, 5.0% of the Staph. aureus infected subgroup and 13.5% of the E. coli infected subgroup had

TABLE 5

Incidence of Inflammation in Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	1	-	8	1	5	1
14	-	1	4	1	8	-
21	-	-	1	-	1	-
42	-	2	10	1	5	2
84	-	-	3	-	6	1
120	-	1	8	0	9	4
TOTAL	1	4	34	3	34	8

[a] Primary closure

[b] Secondary closure

inflamed wounds. Chi-square evaluation showed that differences between these subgroups are not significant (p=.228).

When the incidence of excessive inflammation is compared between the average of all the primary closure subgroups and all the secondary closure subgroups the difference is highly significant ($p=1.32 \times 10^{-5}$).

Analysis of the incidence of abscesses in the primary closure subgroups (Table 6), there was an occurrence of 1.7% in the controls, 26.3% in the Staph. aureus infected animals, and 31.7% in the E. coli infected animals. The chi-square evaluation of this data shows a highly significant difference ($p=1.16 \times 10^{-4}$) between control and infected primary closure subgroups.

In the secondary closure subgroups the incidence of abscess was 3.6% in the controls, 3.3% in the Staph. aureus infected animals and 6.7% in the E. coli infected animals. There is no statistically significant difference between these subgroups (p=.61). A comparison of the incidence of abscesses between the averages of all primary closure and all secondary closure subgroups reveals a highly significant ($p=1.32 \times 10^{-5}$) difference with more abscesses in the primary closure subgroups.

The incidence of hernia (Table 7) in the primary closure subgroups was 5.2% (controls), 10.1% (Staph. aureus-infected animals), and 8.3% (E. coli infected animals). There is no significant difference (p=.61) between these subgroups. In the secondary closure subgroups, the incidence of herniae was 3.6% (controls), 1.6% (Staph. aureus infected animals), and 1.6% (E. coli infected animals). There

TABLE 6

Incidence of Wound Abscess in
Primary and Secondary Closure Wounds in Control,
Staph. aureus-Infected, and E. Coli- Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	1	-	8	1	4	-
14	-	1	2	-	6	-
21	-	-	1	-	1	-
42	-	-	3	1	2	-
84	-	-	1	-	-	-
120	-	1	-	-	6	5
TOTAL	1	2	15	2	19	4

[a] Primary closure

[b] Secondary closure

TABLE 7

Incidence of Hernia in Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected and E. Coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	1	-	-	-	-	-
14	1	-	1	-	-	-
21	-	2	3	-	-	-
42	-	-	-	-	-	-
84	-	-	-	1	-	-
120	1	-	2	-	5	1
TOTAL	3	2	6	1	5	1

[a] Primary closure

[b] Secondary closure

is a significantly (p=.017) lower incidence of herniae in the secondary closure subgroups than in the primary closure subgroups.

Of all animals, there were only three deaths and these all occurred in the Staph. aureus primary closure subgroup (Table 8). Each of these animals, when examined after death, was found to have a necrotic abdominal wall and no other gross abnormalities.

Chi-square evaluation was not done because it would not be satisfactory in view of the low frequency of mortality (3/59) in this subgroup and the absence of mortality in the other subgroups.

D. Scanning Electron Microscopy

Photomicrographs were taken at three powers: first to define the wound, second to demonstrate collagen orientation and third, to demonstrate relative fibre diameter. Representative samples are presented in plate 1. In the control group, a transition from small diameter, disorganized fibres to increasing diameter, coalescent fibres was found. However, no significant differences between the primary and secondary closure subgroups are apparent. A comparison between the control and infected groups likewise, shows no distinguishing differences. The photomicrographs were reviewed by Dr. T. Shnitka of the Department of Pathology, University of Alberta Hospital and by Dr. P. Petrik of the Department of Pathology, Edmonton General hospital. They both concurred with the above.

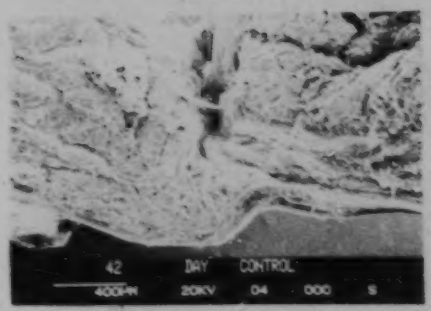
E. Pathological Assessment

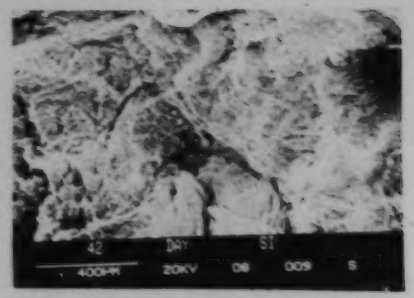
Assessment of the wound tissue was performed by Dr. Peter Petrik of the Edmonton General Hospital. The three slides of each specimen

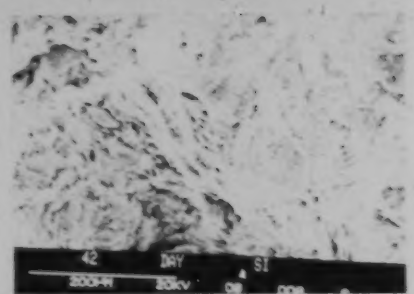
Control
1° Closure
Day 42

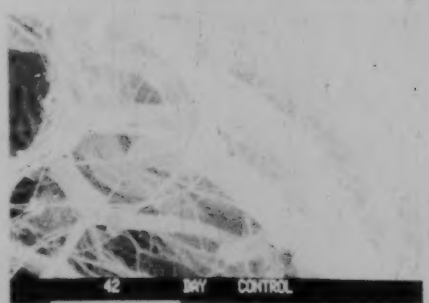
E. Coli Infected
2° Closure
Day 42

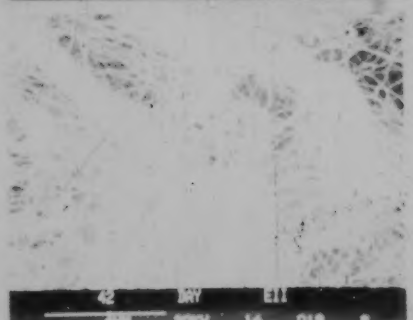
Staph.
Aureus Infected
1° Closure
Day 42











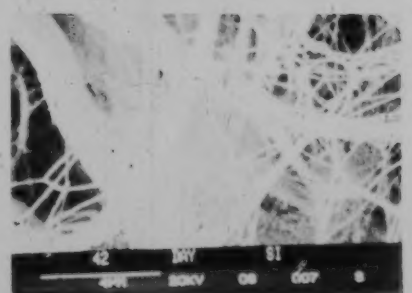


Plate 1

TABLE 8

Incidence of Death Not Related to Anaesthesia in Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	-	-	1	-	-	-
14	-	-	-	-	-	-
21	-	-	-	-	-	-
42	-	-	1	-	-	-
84	-	-	-	-	-	-
120	-	-	1	-	-	-
TOTAL	-	-	3	-	-	-

[a] Primary closure

[b] Secondary closure

were re-labelled prior to examination.

Four aspects of wound healing were reviewed[62]. Firstly, the tissue interposed between the cut surfaces was classified as: granulation tissue, granulation tissue plus immature scar, immature scar or mature scar. These classifications were subjectively separated by the amount of vascularization, inflammatory cell and fibroblast infiltration, and organization and density of connective tissue.

Second, the amount of surface granulation tissue was graded from 1 (minimal) to 3 (maximal).

Third, the collagen of the wound tissue was graded by degree of orientation. One was given for apparent total lack of orientation, 2 for an intermediate stage, and 3 for highly orientated collagen fibres. In addition, content of reticular fibres was graded from 1 (minimal) to 3 (maximal). The pathological review scoring sheet is presented as Table 9.

Fourth, the nature of the inflammatory reaction was described as acute with or without abscesses, or chronic with or without abscesses, or nil. Acute inflammation was characterized by infiltration by polymorphonuclear leukocytes whereas chronic inflammation exhibited a mononuclear leucocyte infiltrate (e.g. monocytes, lymphocytes, macrophages). The degree of reaction was subjectively graded from 1 (minimal) to 3 (maximal). The repair tissue between the muscle edges and that on the wound surface was independently assessed.

TABLE 9

PATHOLOGICAL REVIEW SCORESHEET

TISSUE # _____

I. TYPE OF TISSUE IN DEEP WOUND

- ☐ A. GRANULATION TISSUE
- ☐ B. GRANULATION TISSUE PLUS IMMATURE SCAR
- ☐ C. IMMATURE SCAR
- ☐ D. MATURE SCAR

II. AMOUNT OF SURFACE GRANULATION TISSUE

GRADE

- 1 ☐
- 2 ☐
- 3 ☐

III. INFLAMMATION

		GRADE
A. THROUGHOUT THE REPARATIVE TISSUE		
1. ACUTE WITHOUT ABSCESSES	☐	_____
2. ACUTE WITH MICROABSCESSES	☐	_____
3. CHRONIC WITH MICROABSCESSES	☐	_____
4. CHRONIC	☐	_____
5. NIL	☐	_____
B. ON SURFACE		
1. ACUTE WITHOUT ABSCESSES	☐	_____
2. ACUTE WITH MICROABSCESSES	☐	_____
3. CHRONIC WITH MICROABSCESSES	☐	_____
4. CHRONIC	☐	_____
5. NIL	☐	_____

IV. COLLAGEN

1. ORIENTATION

- 0 (DISORGANIZED) _____
- 1 _____
- 2 (ORGANIZED) _____

2. RETICULAR FIBRES

- 1 (MINIMAL) _____
- 2 _____
- 3 (MAXIMAL) _____

1. Wound Tissue Maturation

The type of tissue in the deep wound was arbitrarily graded as 1 (granulation tissue), 2 (granulation tissue plus immature scar), 3 (immature scar) and 4 (mature scar). This approximates an index of wound maturity and allows for comparisons between subgroups. The sum total in each subgroup was divided by the number of animals in that subgroup. The results are presented in table 10. Each subgroup shows a gradual continuous rise other than the secondarily closed control group at 119 days, at which time two infected wounds influenced the values. The differences in the values between primary and secondary closure are negligible whereas there is significant differences in breaking strength between the two subgroups. The indices of the Staph. aureus subgroups parallel each other except at day 7 when the primarily closed wounds appeared histologically more mature. The E. coli subgroups show similar indices as well as which differs from breaking strength measurements which were significantly decreased at days 14 and 84 in the primary closure subgroup.

2. Surface Granulation

Surface granulation tissue was graded 1, 2, or 3. The average of each subgroup is presented in Table 11. In the control primary closure group, there is an erratic decrease. The decrease is more apparent in the control secondary closure subgroup except that two wound infections in the 119 day subgroup caused exuberant surface granulation tissue and a subsequent rise in the average.

TABLE 10

Wound Maturation Indices of Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	1.3	1.3	2.1	1.0	1.3	1.0
14	2.1	2.3	2.2	2.2	1.7	2.2
21	2.9	2.7	3.1	2.4	2.5	2.9
42	3.2	3.3	3.1	3.6	3.6	3.1
84	3.7	4.0	3.4	3.8	3.7	3.5
119	4.0	3.4	3.8	4.0	3.7	3.9

[a] Primary closure

[b] Secondary closure

TABLE 11

Mean Amount of Surface Granulation Tissue of Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	2.1	2.2	1.6	2.6	2.0	1.9
14	1.3	2.0	1.7	2.2	2.1	2.0
21	1.7	2.1	1.8	1.7	1.8	1.5
42	1.0	1.3	1.6	1.1	1.5	1.8
84	1.3	1.0	1.5	1.4	1.1	1.6
119	1.1	2.1	1.7	1.4	1.6	1.7

[a] Primary closure

[b] Secondary closure

The primarily closed Staph. aureus infected subgroup showed little change with time. At the later dates, however, the averages remained relatively high in relation to both control subgroups (with the exception of the 119 day secondary closure control animals). This may imply a continued inflammatory response. The secondary closure Staph infected subgroup showed excessive granulation tissue at 7 and 14 days with a gradual decline.

The E. coli infected primary closure subgroup shows a gradual decline, much like the comparable control subgroup but unlike the Staph infected subgroup. Interestingly, the surface granulation tissue is at a minimum at 84 days which corresponds with very weak wounds at that same time. The secondary closure E. coli infected animals shows a minimal decrease like the primary closure infected subgroup which once again suggests some stimulus for continued granulation tissue formation.

3. Collagen Orientation and Reticular Fibre Content

The average score for collagen orientation for each date of the subgroups is presented in the second column of Table 12. A value of 1 was assigned to the disorganized grading, 2 to the intermediate grading and 3 to the organized grading. In the control primary closure subgroup there is a continuous gradual increase in collagen fibre orientation which would be expected with wound remodelling.

There is a decrease at 84 days and this can be attributed to two wounds which had both surface and deep tissue chronic inflammation with corresponding intermediate grade fibre orientation. The secondary

TABLE 12

Mean Scores of Collagen Orientation of Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	1.1	1.5	1.8	1.2	1.3	1.2
14	2.0	2.3	2.1	2.1	1.7	1.9
21	2.6	2.4	2.3	2.1	2.1	2.3
42	3.0	2.8	2.7	2.8	2.7	2.6
84	2.8	3.0	2.7	2.9	2.9	2.7
119	3.0	2.7	3.0	3.0	3.0	3.0

[a] Primary closure

[b] Secondary closure

closure control subgroup exhibits a similar tendency to collagen fibre orientation except at 119 days, and again those animals with lesser grades of fibre orientation had coexistent evidence of chronic inflammation and, in one animal, abscess formation. In both subgroups of Staph. and E. coli infected animals there was a relatively constant rise in the collagen orientation score.

The scoring of reticular fibre amount (Table 13) was done in a similar fashion to that of collagen fibre orientation: 1 for a minimal amount, 2 for an intermediate amount, and 3 for a maximal amount. There was a relatively constant reciprocal relationship between collagen orientation and reticular fibre amount (In the control animals in the 119 days secondary closure and the 84th day primary closure subgroups there was a slight increase in reticular fibre amount which was attributable to those animals with histological evidence of inflammation).

4. Inflammatory Changes

The inflammatory changes throughout the reparative tissue and on the surface of the wound were independently assessed. In both instances, the predominant histological characteristic was classified as: acute inflammatory changes, acute inflammation plus miro-abscesses, chronic inflammatory changes, chronic inflammation plus micro-abscesses, or nil (consistent with normal wound healing). These inflammatory changes were subjectively graded as being minimal (1), moderate (2), or severe (3).

The sum of the grades in each histological grouping was

TABLE 13

Mean Amount of Reticular Fibre in Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected Rats, and E. coli-Infected Rats

Days	Control		Staph. aureus Infected		E. coli Infected	
	1°[a]	2°[b]	1°	2°	1°	2°
7	2.8	2.6	2.3	2.6	2.7	2.7
14	2.2	2.0	2.1	2.0	2.8	1.9
21	1.3	1.6	1.8	2.1	2.4	1.4
42	1.3	1.1	1.4	1.0	1.3	1.4
84	1.4	1.0	1.4	1.2	1.4	1.6
119	1.1	1.8	1.1	1.0	1.3	1.1

[a] Primary closure

[b] Secondary closure

multiplied by the incidence of the histological characteristic within the subgroup for that sacrifice date. By this means, an arbitrary degree was assigned to the inflammatory changes. Because of the subjective nature of this method, statistical comparisons are not valid; however, the data in Tables 14 and 15 give an overview of the inflammatory changes of the various subgroups.

5. Wound Surface

On the surface of the control group wounds,, there was a greater degree (i.e. incidence x sum of grades) of acute inflammatory changes present in the early secondary closure wounds. The degrees of acute inflammation plus micro-abscesses were equally low in both subgroups.

The degrees for the chronic inflammatory changes are greater in the early primary closure wound but elevated levels persist in the secondary closure subgroup. In both subgroups, the degrees for chronic inflammation and micro-abscesses were low. There was a greater incidence of non-inflamed wound surfaces in the late primary closed wounds.

On the surface of the Staph. aureus infected wounds, there was a greater degree of acute inflammation and acute inflammation plus micro-abscesses in the early secondary closure wounds. This was in contrast to the greater degree of chronic inflammation, and chronic inflammation plus micro-abscess apparent in the primarily closed wounds. There was a greater incidence of non-inflamed wounds in the secondary closure subgroup.

There was a greater degree of acute inflammation on the surface of the E. coli infected primarily closed wounds. A greater degree of

TABLE 14

Predominant Histological Characteristic and Degree of Inflammation on the Wound Surface of Primary and Secondary Closure Wounds in Control, Staph. aureus-Infected, and E. coli-Infected Rats

Day	Acute(no abscess)[a]		Acute+abscess[a]		Chronic(no abscess)[a]		Chronic+abscess[a]		None[b]	
					Control					
	1°[c]	2°[d]	1°	2°	1°	2°	1°	2°	1°	2°
7	0.2	9.6	2.6	0.3	1.8	0.3	0	0	0	0
14	0	0.3	0	0.3	4.9	0.6	0	0	0.3	0.3
21	0	0.1	0	0	7.8	4.2	0	0	0.2	0.3
42	0	0	0	0	.1	1.6	0	0.2	0.9	0.5
84	0	0	0	0	4.7	3.5	0	0	0.3	0.5
119	0	0	0	0	0	5.3	0.2	0.2	0.9	0.2
					Staph. aureus Infected					
7	.6	7.8	.3	4.8	0	0	4.0	0	0	0
14	0	0	1.0	4.0	4.8	2.0	0	.3	0.2	0.1
21	0	0	0	0	6.0	6.3	.9	0	0.1	0.3
42	0	0	0	0	5.3	.5	.2	0	0.2	.8
84	0	0	0	0	4.0	.4	1.2	.9	0.3	.6
119	0	0	0	0	2.7	.2	0	0	0.6	.8
					E. coli Infected					
7	3.5	1.2	4.4	14.0	.1	0	0	0	0	0
14	1.0	.3	.3	1.3	10.5	.2	0	0	0	.4
21	0	0	0	0	16.2	6.4	0	0	.1	.2
42	0	0	0	.3	9.8	1.2	0	0	.3	.6
84	0	0	0	0	5.4	4.0	0	.3	.4	.4
119	0	0	0	0	2.5	0.2	1.2	1.2	.3	.7

[a] Incidence x sum of grades

[b] Incidence of no detectable inflammation

[c] Primary closure

[d] Secondary closure

TABLE 15

Predominant Histological Characteristic and Degree of Inflammation with the Reparative Tissue of Primary and Secondary Closure Wound in Control, Staph. aureus-Infected, and E. Coli-Infected Rats

Day	Acute(no abscess)[a]		Acute+abscess[a]		Chronic(no abscess)[a]		Chronic+abscess[a]		None[b]	
					Control					
	1°[c]	2°[d]	1°	2°	1°	2°	1°	2°	1°	2°
7	2.0	.8	0.3	0	4.4	10.4	0	0	0	0
14	0	0	0	1.1	7.2	2.9	0	0.3	0	0
21	0	0	0	0	9.8	1.3	0.3	0	0	0
42	0	0	0	0	4.4	4.0	0	0.2	0.4	0.4
84	0	0	0	0	1.8	4.5	0	0.2	0.6	0.4
119	0	0	0	0	0	0.7	0.2	1.3	0.9	0.6
					Staph. aureus Infected					
7	0.1	0.6	0	9.0	9.9	.8	0	0	0	0
14	0	.1	0	.2	1.2	4.8	5.0	1.0	0.2	0
21	0	0	0	0	14.2	13.5	.2	.2	0	0
42	0	0	0	0	6.0	1.1	.3	0	0.2	.6
84	0	0	0	0	3.0	1.3	1.2	0.2	0.3	.6
119	0	0	0	0	4.4	0	0	0	6.4	1.0
					E. coli Infected					
7	0.4	.3	5.0	2.4	.2	.4	0	0	0	0
14	0.2	0	0	.2	20.0	7.7	0	.3	0	0
21	0	0	0	0	12.8	10.4	.3	0	.1	.2
42	0	0	0	0	8.8	8.0	.2	0	.1	.2
84	0	0	0	0	5.6	1.5	0	.3	.3	.6
119	0	0	0	0	1.6	.4	.2	.2	.5	.7

[a] Incidence x sum of grades

[b] Incidence of no detectable inflammation

[c] Primary closure

[d] Secondary closure

acute inflammation plus micro-abscesses was found in the early secondarily closed wounds than the primary closure subgroup. There was a much greater degree of chronic inflammation in the primary closure subgroup and comparable degrees of chronic inflammation plus abscesses in the two subgroups. The incidence of a non-inflammed wound surface was greater in the secondarily closed wounds at all intervals.

6. Reparative Tissue

The reparative tissue was also assessed for inflammatory changes. Little difference in acute inflammation (± micro-abscesses) was present between the two closure subgroups. There was a slightly greater degree of chronic inflammation with or without micro-abscesses) in the secondary closure subgroup and the later primary closure wounds had a slightly greater incidence of non-inflammation.

In the Staph. aureus infected wounds, there was a greater degree of acute inflammation (with or without micro-abscess) in the early secondary closure wounds. The degree of chronic inflammation decreased more rapidly in the secondary closure subgroup and the degree of chronic inflammation plus mirco-abscesses were greater in the primary closure subgroup. The secondary subgroup also had a much greater incidence of non-inflamed tissue in the chronic stage.

The results for the E. coli infected wounds were very similar to those for the Staph. aureus group, except the degrees of chronic inflammation and micro-abscesses were similar in the two subgroups and the difference in non-inflamed tissue was less.

DISCUSSION

A. Animal Model

The choice of secondary closure was determined by the animal model. Delayed primary closure is more often performed clinically but the animals' ability to gnaw at the wound site preclude this type of closure. Although the use of a dorsal wound would eliminate the possibility of oral contact, a change of the experimental incision would then not be comparable to the human celiotomy where delayed closures are most commonly performed. A dorsal wound is firmly splinted by surrounding musculature and is less likely to herniate than the thinner anterior abominal wall wound. Another reason for not choosing the dorsal wound is that it is similar to the human thoracotomy incision, which rarely becomes infected. Thus it seems more appropriate to study the anterior abdominal wound which more readily becomes infected and more often undergoes secondary closure in clinical surgery. Another possible means of protecting the wound would be the use of special dressings. Unfortunately, local experience with wire mesh jackets has revealed that animals are likely to lock their teeth in the mesh and rapidly starve to death. Plaster of Paris jackets proved to be ineffective as the animals were able to gnaw themselves free. A further objection to this form of dressing is that it would introduce a variable in the form of a firm splint which might alter the wound healing process and make the model different from the common clinical celiotomy.

It was apparent that the animals were able to debride their wounds in both the primary and secondary closure subgroups. It is felt that the innoculum bathed the wound for a sufficient time (1-2 hours) with the animal in the supine position for infection to occur. This was borne out by the incidence of inflammation in both the primary and secondary closure subgroups (Table 5). It is consistent with the clinical literature[47-52] that the incidence of inflammation should be lower in the secondary closure subgroups (Staph. aureus 57.6% vs 5%, E. coli 56.7% vs 13.6%). It is to be noted that the clinical use of delayed closures commonly includes forms of debridement (dressing changes and saline irrigations).

Dissection of subcutaneous flaps predisposes to wound infection, however, this technique was employed in all primary and secondary subgroups. The "dead-space" created by the flaps firstly provides for accommodation of the bacterial broth and secondly, may imitiate the significantly thicker, relatively avascular subcutaneous fatty layer of the human which may alsc act as a physiological "dead-space". Although the animals were able to debride these wounds, the model closely approximated the clinical situation of delayed closure and the animals' courses corresponded well to clinical experience.

B. Wound Breaking Strength Measurement

The technique employed gave results which were consistent with the previous literature and the physiological events in the wound. A comparison of the control primary and secondary closure wounds reveals an interesting difference. Secondary closure appears to augment the

strength of the wound throughout the testing period. At day 7, this difference can perhaps be attributed to the excessive granulation tissue, but this morphological feature is not as obvious at 14 or 21 days and by day 42 the skin edges are opposed. It is interesting to note that there is no corresponding difference between the hydroxproline concentration of the control primary and the secondary closure wounds. This implies that there must be a morphological or qualitative difference in the collagen of the secondary closure wounds to account for this difference in strength.

The Staph. aureus infected group showed noteworthy differences compared with the control group[37,42,43]. In the primary closure subgroups, Staph. aureus infection augmented the strength of the wound to levels comparable with those of control secondary closure. Staph. aureus infection and secondary closure do not have an additive effect as both subgroups have similar values. It may be postulated that secondary closure removed the "promoting factor" of Staph. aureus infection, and that the augmentation of wound strength in the Staph. aureus secondary closure subgroup is simply the effect of secondary closure as noted in the control groups.

In the E. coli infected primary closure subgroup, the progression of strength was somewhat erratic. At 14 and 84 days the strength of the wounds was appreciably lower than expected. This might be influenced by the lower incidence of inflammation or abscess formation in the 21 and 42 day groups. These results are reminiscent of the clinical study of Alexander and Prudden[9] who found that wound

disruptions occurring after 11 days were often associated with complicating metabolic factors such as infection. At 84 days, the wound is in a remodelling phase and this time corresponds to the period in which ventral herniae would develop in the postoperative human. It is noted that 5 of the 10 animals in the E. coli infected primary closure subgroup had herniae at this time.

The secondary closure in the E. coli infected animals once again increased the wound strength to levels comparable to those of the secondarily closed control and Staph. aureus infected wounds. It could be suggested that secondary closure negates or prevents the deterimental effects of E. coli infection or that it had an augmenting influence as seen in the control group.

C. Hydroxproline Content

The method of tissue harvesting differed from that used in previous reports[36-38]. However, the changes in hydroxyproline content (Figs. 12-14) correspond to the physiological events, thus substantiating the technique. Hydroxproline content cannot be directly correlated to differences in strength as noted in "Results". Since collagen is the structural element which contributes to wound strength, it must be assumed that qualitative factors rather than simply collagen amount account for the differences in strength. These factors could include fibre diameter, fibre orientation, fibre-bundle (rope-like structure) formation, and intermolecular cross-linking.

D. Animal Outcome

The presence of inflammation is early evidence of a wound infection. There was only one inflamed wound (1.75% in the primary closure control group which is an acceptable rate[63] in a "clean" surgical procedure. In the secondary closure control subgroup, this rate rose to 7.3% and this is most likely due to the closure technique, although the low values make the difference less significant. In the infected groups, the markedly decreased incidence in inflammation with secondary closure is apparent and this phenomena is also found in clinical studies[47-52].

Whereas inflammation is an early sign of infection, the presence of an abscess confirms the infection with localization. Monitoring this aspect gives similar data to that found when inflammation was studied. Secondary closure is associated with a markedly lower rate of abscess formation than primary closure in the infected group, such that the incidence in the infected subgroups is of the same magnitude as that of the secondary closure control wounds.

Wound infection has been found to be associated with wound failure[2,5,6,8,9]. In the control group it is apparent that the type of closure does not affect the rate of hernia formation. In the infected groups, infection with primary closure was accompanied by a

greater incidence of herniation. This may be caused by local toxic factors (acidosis, hypoxia, a bacterial by-product etc.) which would likely be in greater concentration at the wound site in primary closure.

The incidence of death was very low in the study because of the experimental design. It is noteworthy that the only deaths occurred in the Staph. aureus primary closure subgroup. In these animals it appears that the wound infection was a significant factor in their demise. It would appear that secondary closure affords some protection, perhaps by limiting or preventing sepsis.

E. Scanning Electron Microscopy

The scanning electron microphotographs did not contribute to our understanding of the origins of the differences between the wounds. Wound strength gain could be roughly correlated with change (on a continuum) from disorganization with many fine diameter reticular fibres to an organized structure with large diameter fibres. A 1 mm length of wound was selected from 7 cm of incision in one of 10 animals per subgroup. This small sample may not have been representative of the subgroup and therefore differences in transitional changes were not distinguishable. The SEM best lends itself to the detection of the presence or absence of a morphological feature whereas transitional changes are difficult to appreciate.

F. Pathological Asessment

1. Wound Tissue Maturation

Numerical assignment of wound maturity has obvious subjective limitations, however, patterns and progressions can be discerned. In the control group, unknown infections in the 119 day secondary closure subgroup were felt to be responsible for the decline of the index. It is interesting that these infected wounds did not progress to a state of "mature collagen" as seen on light microscopy. Additionally, collagen orientation was relatively decreased and there was a relative increase of reticular fibres. It is possible that the infection may have "kept" the wound healing process partially in an inflammatory phase or that the infection may have had an inhibitory effect on the subtle mechanisms of the remodelling phase. Because of the design of the experiment, these microscopically infected wounds were not appreciated until a later date and SEM was not possible.

In the Staph. aureus infected wounds, the indices of the two subgroups are relatively predictable. The primary closure 7 day subgroup has an index which is comparatively greater than any other 7 day subgroup. When the inflammatory changes of this particular subgroup are examined, the value for chronic inflammation in the reparative tissue is much greater than all other 7 day-subgroups for

chronic inflammation plus micro-abscesses on the surface of the wound is greater than the remainder of the 7 day subgroups except for the 7 day secondary closure control wounds. In this particular instance the Staph. aureus infection may have had a stimulatory effect on collagen and ground substance synthesis; however, this change is not reflected in any increase of wound breaking strength.

The maturation indices of the E. coli subgroups demonstrate a gradual increase with time; but no correlation with wound bursting strength. The relatively parallel increase in maturation does not correspond with the disparate changes of wound breaking strength of the two subgroups.

2. Amount of Surface Granulation Tissue

There were two irregularities in the relatively constant pattern of decline with time. First, it was noted that there was an increased amount of granulation tissue in the 119 day secondary closure subgroup and this corresponded with two perisistent infections in this subgroup. No alterations of wound breaking strengths in these infected "control" wounds were apparent. Second, a corresponding decrease of surface granulation tissue and wound breaking strength is noted in the 84 day primary closure E. coli infected wounds. It might be conjectured that the surface granulation tissue affords additional strength to the wound by a direct mechanical effect. The relative absence of granulation tissue may also provide a less favourable

physical envirnoment for the maturation of the wound collagen. It may be speculated that the granulation tissue elaborates "factors" which promote wound healing.

3. Collagen Orientation & Reticular Fibre Amount

These two factors showed a relatively constant reciprocal relationship but unfortunately their relative changes did not correlate with wound breaking strengths. It may be that there is a more subtle change of collagen morphology or composition that is responsible. It is generally agreed that reticular fibres correspond to type III collagen, and that type III collagen acts as a matrix for further collagen deposition[22,30,31]. Perhaps alterations of a "core" type III collagen, or type I-III interaction are responsible for relative mechanical property differences.

4. Assessment of Inflammatory Changes

Review of the histology slides indicated that both surface and deep wound tissue warrant separate assessment. This is a factor of the excessive granulation tissue which normally accompanies secondary healing. It is recognized that normal wound healing is accompanied by an inflammatory response and as well that, in cross-section, fibroblasts are difficult to distinguish from the mononuclear inflammatory cells of chronic infection. It is therefore conceded that the scoring system does not represent absolute but rather relative values for the sake of comparison. Statistical evaluation of such data is not valid.

In the control group, an acute inflammatory response is more pronounced in the surface of the early secondary closure subgroup. This is not surprising, considering the larger wound volume which would require debridement. Micro-abscesses are slightly more prevalent in the reparative tissue of the secondary closure subgroup. In addition, there is a greater tendency for normal (i.e. non-inflammed) healing to occur on the surface and in the depths of the primary closed wound. This is not unexpected as primary closure in the clean surgical wound allows for protective skin dressing to prevent any further contamination. It is interesting to note that the occurrence of wound abscesses and inflammation is comparable between the control and infected secondary closure subgroups. It appears that secondary closure is a helpful adjuvant in the contaminated wounds but it does not completely abolish the gross or microscopic sequelae of infection in clean or contaminated wounds.

In the Staph. aureus infected group, the predominant trend is one of a persistent chronic inflammatory response and a greater incidence of a pathological inflammatory response in the primary closure subgroup. This correlated well with the clinical outcome of the animals but has no relationship to the wound breaking strengths or hydroxyproline concentration of the wound. It is possible that the Staph. aureus innoculation concentration resulted only in an augmented inflammatory response as autogenous blood did in the work of Myers and Rightor[25] and that there was a satisfactory immune response by the

animals such that the wound healing "module" was not arrested by overwhelming local septic complications.

It is possible that these local septic complications may have co-existed with an augmented healing process. As previously discussed, the samples for wound breaking strength were deliberately not taken from an area of a wound complication. Therefore, the active Staph. aureus infection which persited beneath primary closure may have had a non-uniform effect. The mechanism of augmentation of wound breaking strength was not determined in this study.

The histological evaluation of the E. coli infected group revealed minimal differences of inflammatory response between the primary and secondary closure subgroups. This is not consistent with the differences found in wound breaking strengths. It is possible that the organisms in the primarily closed wounds release some factor(s) that affects the synthesis or organization of the collagen, giving a poorer quality wound.

The possibility of a weaker wound collagen is intriguing, as acute wounds are often splinted by voluntary and involuntary local muscle contraction. With time, the wound becomes less painful, and the reflex muscle splinting lessens, thus an infected wound with a poorer quality collagen would be more likely to fail with the stresses of late convalescence.

CONCLUSION

In summary, this thesis has delineated several characteristics of the secondarily closed wound. It confirms the clinical reviews[47-52] and previous experimental studies [45-46] that secondary closure of contaminated wounds results in a decreased incidence of wound complications.

This study examines secondary closure wound breaking strengths which has not previously been undertaken. In clean wounds, secondary closure has been found to augment the fascial wound breaking strengths both acutely and chronically. This information reassures the clinician that the strength of his patient's wound would not be jeopardized by secondary closure.

Assessment of secondary closure in this study advances the understanding of wound healing in an infected environment.

The noted augmentation of strength of secondary closure may be of considerable importance to states of deficient wound healing. It is apparent that the unknown effector mechanism and functional or structural cause of wound strength augmentation merit further study.

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